

8 October 1981

What European policy on science?

The appointment last week of Professor Paolo Fasella as Director-General of Research, Science and Education in Brussels is a surprising reminder that the European Community also has a stake in making policy on technical matters. Surprising? Because it is almost a decade since the Community did anything effective in this field (which does not mean that it has ever failed to keep the Joint Research Centre at Ispra in Italy supplied with funds). So why should the accession of Professor Fasella be taken as a sign that things may be different in the future? Because Fasella is an energetic man with a passion for building bridges between the universities and industry, because the community is coming to a point in its brief history — a mere quarter of a century — when an effective policy in regard to science and technology would be feasible and also because if Fasella, like his predecessors in Brussels, gets nowhere, that will be a sure sign that the community should give up the struggle, leaving policy in the field to member governments.

There are also two chief underlying difficulties, the most serious of which is constitutional. The European Commission is neither a government nor an independent agency, but a kind of judicial body empowered to implement the Treaty of Rome (which means that it can be tough about trade protection) but otherwise given a licence to function only when no substantial member government dissents. Since the treaty has nothing specific to say about science and technology (even though quite a lot about nuclear energy, a hangover from the Euratom Treaty), successful initiatives require the support of member governments. This is why, more than a decade ago, M. Pierre Aigrain's ambitious schemes for the creation of a European computer and communications industry came to nothing; most governments were opposed to change.

The other difficulty that Professor Fasella will run up against in Brussels is that the European Commission, although widely reviled as a gigantic bureaucracy, lacks many of the rudimentary facilities governments have at their disposal. People may be well-paid by national standards, but there are only handfuls of them, not often distinguished intellectually or as administrators. Advisory groups, expected to reflect a kind of national balance, are too impermanent to be of substantial help to those in charge of policy. And, in the competitive hothouse of Brussels, directors-general have an incentive to strike out in adventurous, even noisy, directions rather than to ensure that the Community's administration of science and technology is conducted sensibly. Professor Fasella will be well advised if, for a time at least, he can stay out of that rat-race.

But what else is there to do in Brussels? Unenviably, the Community has acquired in the past decade or more a reputation for naivety in technical affairs that would cause even the smallest local authorities to hang their heads. In devising policies for regulating pollution, the manufacture of toxic chemicals or even the allocation of fishing rights in Community waters, the commission has often behaved like a bunch of lawyers seeking to ensure that everything will be for the best in some unreal world. The most urgent task, for Professor Fasella, is somehow to ensure that the commission's routine regulatory work, its bread and butter, is technically competent. That is far from easy — the directorates at Brussels are notoriously balkanized. Directors-general seem mostly to be bound by the convention that they should not seek to influence each other's affairs. Professor Fasella should avoid that trap.

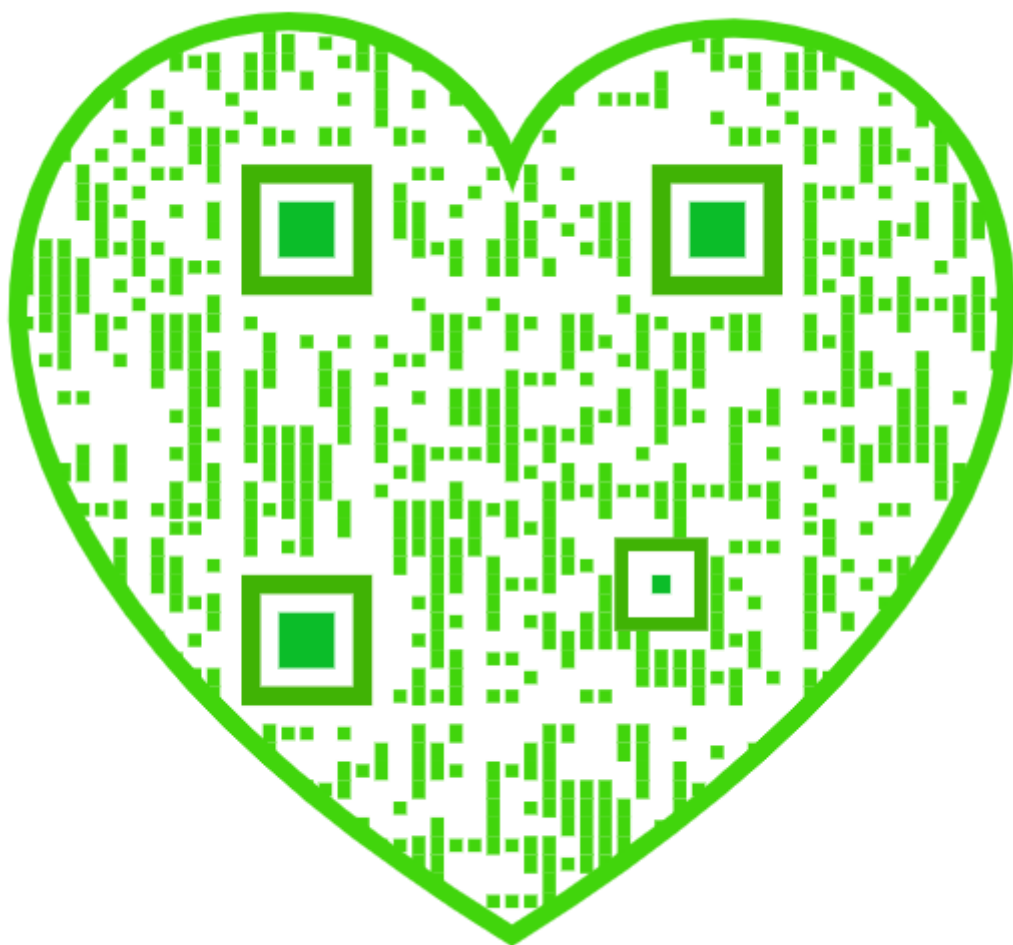
He should also avoid the trap of the spectacular initiative — some great plan for the reinvigoration of European metal-forming technology, submarine cargo transport or even airship technology. Such schemes have foundered in the past, and will continue to founder, on the jealous self-interest of member governments, to whom collaborative projects are attractive only when they are sure of being able to recover at least as much money (by way of contracts for their own nationals) as they contribute. (The European thermonuclear project JET is an exception only because its possible application lies some way ahead.) In the long run, the only stable solution is that governments should agree that contracts for public purchases should be made competitively. (At present, the Treaty of Rome excepts governments from the rules of competition that apply to private companies and individuals.) It would be asking a lot that a mere director-general of research, science and education should solve that problem single-handed. Professor Fasella should, however, keep it in mind.

For the rest, Professor Fasella's most constructive work will seem quite humdrum. He, and the Community, must take an interest in the wellbeing of the scientific community. The encouraging signs, in the 1960s, of a growing sense of integration within European science faltered in the 1970s, as national governments ran out of the funds with which to pay for *ad hoc* schemes. There is now a danger that collaboration between European scientists will be further restricted. The Community has an interest to ensure that this does not happen, and the commission has a duty in the same direction. Quite modest funds would help. But instead of working through cumbersome *ad hoc* instruments (or even the apparently useless committee called GERD), the commission should work directly with national research councils, perhaps in the form of the European Science Foundation. Professor Fasella should cut his teeth on modest tasks like that.

Off with their heads!

President Reagan did not, as is now widely supposed, abolish the Departments of Energy and of Education in his television broadcast two weeks ago. Instead, he said that he proposed that they should be abolished. What will now happen is that the White House will prepare plans for deciding how the statutory obligations of the two departments should be transferred elsewhere (or perhaps even liquidated). Congress will then say whether it approves the plan — and in the process there will no doubt be an unholy fight about the propriety of what is proposed: the Department of Education was, after all, one of President Carter's concessions to liberal opinion. Then, two or three years from now, the two departments may go out of business. President Reagan thus reaped the benefit of practising what he was preaching last November — the benefit of small government — but neither department has disappeared yet.

It is not even clear what plans the White House will be putting forward. The Department of Energy, which did not exist eight years ago, will not be unscrambled easily. For the department has been saddled with responsibility for the torrent of legislation on energy matters that Congress has been passing in the past few years including the Energy Act of 1977 which, *inter alia*, solemnly declares that "this Congress considers that the bicycle is the most economical form of transport". The department superintends the regulation (or deregulation) of prices for oil and natural gas,



receives applications from private individuals wishing to build their own hydroelectric plants, monitors progress towards energy sufficiency, tells oil refineries how to operate, stockpiles strategic oil reserves and, partly as the residual legatee of the operational part of the old Atomic Energy Commission but also because of the wish-fulfillment of successive congresses and presidents, administers a research budget of \$2,000 million. The danger is that in unscrambling this omelette, the White House will sacrifice on ideological grounds those parts of the department's work that cannot be moved about like the pieces in a board game.

The research budget is the most vulnerable. Most of the administrative functions specified by legislation could probably be transferred, without ambiguity, to the Departments of Commerce or of the Interior in such a way that nobody would know six months later that there had been a change. The department's research will need gentler handling, even though the case for dismemberment is stronger. For it has always been anomalous, the result of an historical accident, that first the Atomic Energy Commission and now the Department of Energy should be responsible for essentially academic research in high-energy physics. The natural home for this function is the National Science Foundation; for is not high-energy physics part of basic research? This proposal will not be welcome to the high-energy physics community, by now well used to the department's avuncular ways. But they are likely to be safer with the foundation than with the other agencies to which they might be shuttled. For them, there is either the frying-pan or the fire.

The other components of the department's research budget are in for a rougher ride. Already the Administration has made it plain that the ambitious Carter plan for research and development (by publicly supported demonstration) in solar energy will be drastically cut back, on the grounds that those parts of programmes likely to yield short-term benefits should be able to stand on their own feet. Basic research in, say, photovoltaic processes is unlikely to grind to a halt — the service departments will probably take up the slack. But environmentalists will be up in arms. With this precedent for solar energy, however, the White House planners will have an awkward decision to make on nuclear energy. It will be easy to suggest that the weapons laboratories at Los Alamos and Livermore should go to the Department of Defense, but should there be public support of any kind for a technology as well advanced as the civil application of nuclear energy? Both the ideologues of small government and the environmentalists will make hay of that.

The difficulties the Administration will encounter on such matters are self-inflicted. On solar energy, the Administration would have been on sure ground if it had said, earlier in the year, that Mr Carter's legacy of solar energy research was too ambitious, too unrealistic and too costly, and that substantial trimming would be necessary. Instead, the Administration seems to have put its tongue in its cheek (and hoped to head off a fight with Congress as a result) by saying that the time has come when industry should take up the slack. This is disingenuous. Even if the assumption were true, there would be a case for continued government investment in the basic research and technology of, say, direct electricity generation from sunlight. The same is true of the civil nuclear programme. Does the United States want to be stuck for years to come with those systems that manufacturers are able now to offer? Paradoxically, and inconsistently, the Administration seems to have accepted this argument for the programme for converting coal into synthetic fuels, and has agreed that its demonstration programme should continue.

The moral, for the disbandment of the Department of Energy, is that the Administration should be careful that it picks up the essential pieces if and when the department is closed. The proposals to disband the Department of Education will be more contentious but will raise fewer substantial issues. Given the principle that education at all levels is the responsibility of private institutions, it is outwardly consistent that the federal government should withdraw. But is it proper that the Administration should now pretend to wash its hands of responsibility for education? And does it intend, in the process, to transfer responsibility for

the truncated programme of student loans to the private banking sector? Will it give up the fight on engineering education, now hamstrung by the competition from industry for potential academics? In the old days of the Department of Health, Education and Welfare, such issues could be followed up provided there was a strong man in charge of the Office of Education. Now there is no natural home for such an agency. The Administration may find it necessary to invent one.

Monuments for whom?

The Department of the Environment is well on the way to making a muddle of its policy on British ancient monuments and on Stonehenge in particular. Quite properly, the department is anxious that the monuments should not deteriorate still further as a consequence of their popularity with summer tourists. For if the mountain paths in highland Britain are in danger of being eroded by the hobnailed boots of those who must now in the summer holidays queue for a place in the line of those clambering up the popular spots, the threat to more accessible places such as Stonehenge is evidently great. The trouble is that the department's policy appears to be based on the assumption that all visitors to these places are potentially vandals. The result is that even access by archaeologists to the monument at Stonehenge is being controlled as rigorously as if these learned people were likely to paint their names with spray-cans on the standing stones once the custodians' backs were turned. Briefly, parties of archaeologists must apply in advance to the Department usually allowed to visit it for an hour in the early morning on Tuesdays only, before the public is admitted to the site at 9 a.m. (but all day during the winter).

By any standards, this policy smacks too much of bumbledom. The chances that serious academic people will be able to carry out serious work during such brief intervals are negligible. The department may fear that members of the public (who are now kept some distance from the site by a ring fence) might be maddened beyond restraint by the sight of academics given closer access and even the opportunity to turn a spade, but that is a poor excuse. So too is the ready calculation that more access by archaeologists would mean a larger wages bill to cover the cost of supervision that is not, in all the circumstances, strictly necessary. For serious archaeologists, the present restrictions are all the more galling because of the department's apparently willing agreement that members of the whimsical order of druids should be allowed each summer solstice to conduct a ceremony on the site in a laughable attempt to perpetuate the ceremonies supposed to have been enacted at Stonehenge some years ago. And while nobody disputes that the site at Stonehenge must somehow be protected, few hearts leap up at the sight of the car park, the public lavatories and the money-making snack bar with which the department has graced the sight — admittedly below eye-level.

In the circumstances, the most urgent need is for a coherent policy by means of which the archaeological and public interests in important sites can cheerfully coexist. The department does indeed support a Commission on Ancient Monuments, to which advisory committees of archaeologists are entitled to make their case. What seems most of all to be lacking is imagination. There can be few circumstances in which members of the general public would not be enlivened to know what steps are being taken to make plain the significance of these important sites. And even at places such as Stonehenge, there is no reason why investigations should not continue throughout the day (and even night). But an accommodation between the professionals and the public would be most easily arranged if some of the modern structures on the site, the work of the department and not the original builders of the monument, were given over to a small museum that would describe what is known of Stonehenge and what is being done to deepen this understanding. As things are, the most evocative monument in Britain could become just a picnic place on one of the ancient routes across Salisbury Plain. That is a shabby fate for the first of all publicly supported research establishments.

Conflict of interest on California campus

Davis scientist steps away from grant

Washington

In a move that again reflects tensions between academic and commercial interests in biotechnology, a prominent plant geneticist at the University of California's Davis campus has agreed to step down from a multi-million dollar sponsored research contract financed by a large fertilizer company because of a potential conflict of interest with a private research company in which both he and the fertilizer manufacturer are also involved.

Dr Ray Valentine, internationally known for his research on the isolation of the *nif* genes present in plant-colonizing bacteria and responsible for the nitrogen-fixing properties of some plants, has also offered to resign from the university's Agricultural Experiment Station, where the sponsored research would be carried out. Although remaining on the faculty, where he is professor of plant biology, such a move would increase his responsibility for undergraduate and postgraduate teaching and reduce his involvement with university research programmes.

Dr Valentine's offer to restrict his university-based research follows concerns raised by Dr Charles Hess, dean of the university's College of Agricultural and Environmental Sciences, over the implications of a five-year, \$2.3 million research contract signed in July with Allied Chemical Corporation, for which Dr Valentine was to have been a principal investigator. The research under this contract would explore ways in which the bacterial genes discovered by Dr Valentine might be transferred to other plants to enable such plants fix their own nitrogen.

A few days after the deal with Allied Chemical Corporation was signed, the company announced that it had agreed to purchase a 20 per cent equity interest, at a cost of \$2 million, in a Davis-based company called Calgene which Dr Valentine had helped to set up and of which he is a vice-president.

On learning this news, Dr Hess told Dr Valentine that, as a result of concern among faculty members about potential conflicts of interest he would either have to withdraw from his involvement in the Allied Chemical grant to the university, or from Calgene. Dean Hess also announced that he was holding up the company's grant until an acceptable arrangement had been made.

Dean Hess has suggested two possible statements of principle which the faculty might use as a framework for establishing

outside contracts. One would be that individuals with appointments in the Agricultural Experiment Station "shall not accept gifts, grants or research contracts with private firms in which they have an equity interest or serve as a consultant" — a condition that would have applied to Dr Valentine's case. Dean Hess has also suggested that such individuals "shall not have an equity interest in private firms whose research programs are the same as the individual's Experiment Station research programme".

Emphasizing his complete confidence in the integrity of Dr Valentine, Dean Hess said that as the college and experimental station were funded primarily from public sources there was also concern about "whether these apparent conflicts of interest might jeopardize the support that we get from tax funds if people began to feel that the return from the research was being realized by private investors".

Calgene officials for their part have

denied that there would necessarily be a conflict of interest. Although major funding has been received from Allied Chemical, they insist that their main research interest will be on how to improve the efficiency of fertilizers, rather than looking at plants themselves, as researchers at the experiment station will be doing.

Faculty members at Davis will meet next month to discuss Dean Hess's proposals. Meanwhile Dr Valentine's future relationship with the university remains uncertain. Dean Hess says that he intends to "discuss" Dr Valentine's offer to resign from the experiment station completely rather than just withdraw from the Allied Chemical project. The university is keen to avoid any public criticism about conflicts of interest. At the same time, it is reluctant to put off potential corporate sponsors of its research which are becoming an increasingly important source of funding as federal and state financing for research begins to contract.

David Dickson

Technology assessment wins new friends

Washington

Two years off its tenth birthday, the United States Congress's Office of Technology Assessment (OTA) at last seems to be coming of age. By adopting a more pragmatic outlook OTA has generated a political and scientific credibility that makes its prospects more secure than ever before.

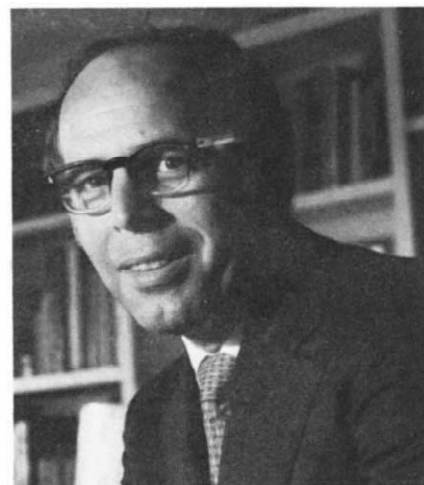
The change has its critics. "OTA is turning out good craft pieces that serve the interests of clients, but there is still a latent demand for something which is more policy- and futures-rich" says Dr Joseph Coates, who was among the original staff members and recently left to establish his own consultancy firm.

But evidence of the new-found stability, which broadly coincides with the reign of OTA's present director, Dr John Gibbons, previously head of the Energy, Environment and Resources Center at the University of Tennessee, was shown last week by the willingness of a Republican-dominated Senate, in a time of reduced federal spending, to increase the OTA's budget by 7.5 per cent next year to \$12 million. Even though this will barely allow OTA to keep up with inflation, it compares with the budget to which the office has been held between 1980 and 1981.

When OTA was established by Congress in 1972, finally coming into being in November 1973, many saw it — with some justification — as a vehicle through which Democrat legislators hoped to challenge the scientific and technical decisions of the Nixon Administration following the demise of the Office of Science and Technology and the President's Science Advisory Committee.

OTA's new-found friends, however,

include conservative Republicans such as Senator Paul Laxalt of Nevada. Senator Laxalt is said to have been impressed by a recent study carried out by OTA comparing different basing modes for the MX missile which point out the advantages and drawbacks of each without endorsing a



Dr John Gibbons — forging links

particular design, issues directly pertinent to the debate about placing the missiles in his home state.

The importance of such friends emerged two weeks ago, when they helped to head off an attempt by Senator Mack Mattingly, chairman of the Senate appropriations subcommittee responsible for legislative affairs, to kill future funding for the OTA on the grounds that its studies duplicate the work of other bodies such as the Congressional Research Service.

It was a charge that was strongly denied by Dr Gibbons in hearings on the OTA

budget earlier this year. He pointed out that the cases quoted by Mr Mattingly — for example of consulting firms carrying out the same work for different agencies — were several years old, and that with a tighter management structure and more narrowly defined missions, OTA was now beginning to demonstrate unique value to Congress.

Much to OTA's relief, Senator Mattingly did not take his case to the floor of the Senate, and OTA's 1982 budget is now secure. "I presume it did not come up because a lot of people on both sides of the aisle have come to see OTA as a useful and important analytical tool", said Dr Gibbons last week.

Much of the recent work, both long-term and short-term, has impressed OTA's congressional clients. "Some of their stuff is really excellent, particularly in the energy field" says one staff member of the House Science and Technology Committee. A recent report, *Impacts of Applied Genetics*, an analysis of the economic and commercial implications of genetic engineering applied to microorganisms, plants and animals, has been widely praised and recently reached the "top ten list" of government publications.

One secret of OTA's survival seems to be its greater awareness of the limits within which it can safely operate. The genetic engineering study, for example, avoided all discussion of the human applications as "beyond its scope", and the MX study, while studying the "socio-economic" implications of the weapons' deployment, did not discuss political factors such as the full implications of local resistance. Dr Gibbons explains this pragmatically. "Our job is to narrow the issues that must be fought out rather than to tell the politicians what they should do", he says. He talks of breaking problems down into "bite-size" pieces, referring, for example, to recent OTA studies on energy utilization and demand.

Dr Gibbons is currently trying to forge closer links with the National Academy of Sciences, as well as discussing ways of complementing the policy orientations of the Office of Science and Technology Policy. Drawing technology assessment more closely into the traditional nexus of political decision-making may have disenchanted some of its earlier enthusiasts. But it has proved to be a formula for political survival.

David Dickson

Islamic science

Radicals agree

Islam and a handful of radical Western scientists came together in a royal palace last week, leading to what one Islamic scholar called a "breakthrough in Islamic studies".

The objective of the meeting was to develop a critique of the role of science and technology in society as seen from an

Islamic perspective and using Islamic values. This is not a matter of counting Islamic angels on a needle point, but an issue central to the 42-nation Muslim world seeking both to absorb the fruits of science and technology without too much social disturbance, and to rally behind the flag and philosophy of Islam. The study was the first in a series of science in Islam and the West being organized by the International Federation of Institutes for Advanced Study (IFIAS), set up by the Nobel Foundation to consider issues of academic weight and world importance. King Gustav of Sweden has given the federation a palace as headquarters and conference centre.

Inspiration for the conference came from Ziauddin Sardar, a young Londoner born of Pakistani parents (and one-time correspondent of *Nature*) who has a passion for Islam and the issues of development in the Muslim world. He appears to have achieved a rapprochement between Westerners and the senior and devout Muslim scientists at the meeting.

Initially the meeting seemed likely to founder on the rock of the Muslim insistence on the purity of any search for knowledge. Islam directs the faithful to seek knowledge of the natural world, and inspiration in it: the world is the work of God. This concept, while appealing to Western scientific egos, seemed incomplete to the radicals — to whom some kinds of scientific knowledge were like a "second bite of the apple". This was heresy to the Muslims.

Distinctive development

The delicacy of politics in the Muslim world helps to explain why it has taken three years to install the director-general of the Islamic Foundation for Science, Technology and Development, Dr Ali Kettani, in the Jeddah headquarters.

Kettani has to deal with 42 diverse Muslim nations embracing Africa, the OPEC states and the Far East — each with its own internal political problems, its own approach to Islam and its own goal in "development". Kettani's goal is to forge an Islamic brotherhood of scientists and technologists, who would turn to each other, rather than to the West, to solve problems.

The foundation has been promised an initial \$50 million for 2–3 years recommended by the Organization of the Islamic Conference (of which \$15 million has already been paid by Saudi Arabia). There is to be a staff of some 30–40 and a charter which requires the foundation "to ensure that all member countries . . . both individually and collectively, make the greatest possible use of science and technology (including the social sciences) in the formulation and implementation of their socio-economic plans, keeping in mind the need to consolidate the unique Islamic personality and character".

The Westerners would also not allow a firm distinction between the search for knowledge and its use, which would have solved the dilemma. The idea was simplistic, they said: half the world's science is attributable to defence and industrial research budgets, even if that science is, in its minutiae, "fundamental".

The solution came from Dr Ali Kettani, recently appointed director of the Islamic Foundation for Science, Technology and Development (see box). In Islam, both the ends and means of an individual's actions must be "hallal" (allowed), so knowledge sought within a context of "haram" (forbidden) use would also be forbidden.

The social context and consequences of science could also be considered within Islam, the meeting judged, using precisely defined Quranic concepts such as "adl" (equity), "zulm" (oppression), "muslah" (public interest) and "dyah" (waste).

A measure of the success of the meeting was that the participants tore up their papers, prepared before the conference, and which were to have been the basis of a book, promising to rewrite them in the light of what they had learned. Subsequent meetings in the seminar series will turn to more practical technical issues, such as energy and environment (to be held in Riyadh), habitation, agriculture, health, and industry and mass production.

Robert Walgate

London science centre

No cash ahead

The new London Science Centre is appealing for money to keep going for a second year. The centre was established last April by the Foundation for Science and Technology with about £50,000 of donations from learned societies and industry. It is now looking for a further £200,000 to tide it over until it becomes self-financing.

The centre's aim is to provide facilities for small learned societies whose existence is threatened by the high cost of central London. So far, interest has been modest. Only twenty-six societies have joined, and are entitled to pay less than the going rate for assistance with their day-to-day administration. The main facilities on offer are two small conference rooms and five offices, together with secretarial assistance, at the centre's rented headquarters in the Royal Society of Arts.

The centre's preoccupation during its first six months has been to take over the role of liaison among learned societies from the Royal Society. But plans for the future are more ambitious. The idea is that the centre will not only offer facilities such as word-processing and computerized membership lists, but will become a focus for interdisciplinary communication.

Even so, its centre is the residue of a far more ambitious scheme to emulate the Clunies Ross Memorial Foundation set up

in Australia in 1963. With the backing of government and industry, that was meant to provide Australia with a prestigious scientific meeting place. Its success prompted the Commonwealth Foundation to sponsor similar centres in twelve other capitals. But the attempt to set one up in Britain failed, largely because of the existence of well-established learned societies.

The idea was nevertheless kept alive by a group of senior scientists and administrators, including the president of the Royal Society and the chairmen of the science and medical research councils, concerned to help small specialist learned societies out of financial difficulties. In 1977, the group set up the Foundation for Science and Technology with the remit of raising funds to set up small science centres in British cities.

The London Science Centre is the first. The foundation is now in the throes of appointing a permanent director to take over from its interim director John Chadwick, previously director of the Commonwealth Foundation. But the more immediate problem is to raise enough money to ensure the centre's existence beyond next March.

Judy Redfearn

World wildlife fund

Bank on conservation

This year the World Wildlife Fund (WWF) is celebrating its twentieth anniversary, and something of a revolution in its policy and strategies. While its aim is still the preservation of the world's endangered flora and fauna, the old incompatibility with the demands of development in the Third World has been resolved. The World Conservation strategy, now in circulation for 18 months, shows that there can be no conservation without ecologically and environmentally sound development policies.

How far this conviction has gained ground within the policies of the European Community was brought to light in a seminar held in Brussels last week, between the European Commission and international conservation organizations. Although the renegotiated treaty between the European Economic Community and the sixty-one African, Caribbean and Pacific (ACP) countries (Lomé II), which are largely old European colonies, now includes a reference to the need for environmental considerations in development projects, the reality in the field belies this exhortation.

The recently completed report by the International Institute for Environment and Development (IIED) on "the European development fund and environment" reveals in case studies undertaken in Jamaica, Guyana, Burundi and Mali the poor degree of environmental impact planning. Failure to consider environmental factors in the planning of develop-

ment projects has led to the failure of projects, and produces projects which themselves cause environmental damage and the loss of valuable resources.

Although national governments have paid lip-service to the development strategies outlined in the Brandt report and the World Conservation Strategy, the IIED report suggests that the government agencies responsible for aid management have yet to take them seriously.

Speakers at the seminar in Brussels claimed that European Community member states themselves do not pursue economic policies based on the sustainable use resources, and that the Common Agricultural Policy is an instrument for environmental damage not only at home but abroad too. Developing countries are being encouraged to misuse their resources to support irrational pattern of agricultural production and consumption in Europe.

European Commissioner for environmental affairs, Karl-Heinz Narjes, agreed with the need for agricultural and development policies in line with the principles of the World Conservation Strategy. But he pointed out that the Commission and European Parliament were still wrestling with the member states even to win the modest finance needed for an environmental fund from the EEC budget. The prospects for finding the money to expand the work of the commission to include environmental planning were poor.

The need for agricultural research to devote more time to developing agricultural techniques which are also ecologically sound was also discussed at the seminar. A 110-page study just released by the World Bank calls on developing countries to step up their investment in agricultural research from an average of 0.31 per cent of agricultural gross domestic product (1975) to 2 per cent. The number of research scientists, says the bank, will need to increase by 9,000 by the year 1984. Such an ambitious programme would establish a solid base in research and would be an important step in helping to meet the food needs of developing countries.

The World Bank itself plans to expand its lending for agricultural research from \$350 million in fiscal year 1981 to approximately \$550 million in 1984. The lending for research and extension in 1984 will be 13 per cent of the \$4,600 million the bank lends for agricultural and rural development — up from 9 per cent in 1980.

Jasper Becker

Science parks

Plans forged

A few British universities are planning to set up science parks on the pattern of Research Triangle in North Carolina; the example of route 128 around Boston is less attainable. Although plans for the parks began before the latest crisis, universities hope that the parks will earn them at least

some extra income.

Local authorities, especially in areas of high unemployment, are enthusiastic, seeing the parks as a means of attracting new high technology industries and more jobs. Some of the latest schemes differ from the two existing parks attached to British universities — a research and development park at Heriot-Watt and a science park at Cambridge — chiefly by the support they have won from local government.

So far, the most advanced scheme is that announced last week by the council of the City of Birmingham, the second largest city in Britain. The city plans to spend £2.5 million on a science and technology development centre attached to the University of Aston in Birmingham, one of those whose budgets were sharply reduced by the University Grants Committee. The university has since been looking to industry for moral if not financial support.

The city money will pay for the refurbishment of a 3.5-acre inner city site next to the university to provide units for small manufacturing firms or research and development laboratories. More land is to be made available when companies need to expand. The city hopes that new, small companies as well as established multinationals will be attracted to the site, helping the university by paying for the use of facilities and expertise.

The hope is that academics will take on more consultancies and will even be encouraged to set up their own small firms, although the arrangements whereby they can spend up to one day a week working for industry will not be changed. Secondment for those involved in establishing firms, however, will be looked on favourably, according to Professor Frederick Crawford, vice-chancellor of the university.

The initiative for the Aston park came from the city council, hitherto persuaded that neither of its two universities, Aston and Birmingham, was capable of collaborating effectively. However, the appointment of Professor Crawford, who has been involved with the Stanford Industrial Park in California, as vice-chancellor last year, seems to have changed that opinion.

The city council of Salford, an industrial city in the depressed north-west of England, is planning a similar collaboration with its local university, also severely penalized by the grants committee. The council spent £250,000 last year on establishing a pilot science park large enough to house four small technological companies. It has sold land in the Salford enterprise zone (where companies are exempt from rates and planning permission) to a commercial company which will open a larger science park next month. The new park will have close links with Salford University Industrial Centre Limited, a commercial company set up by the university 12 years ago to forge links with industry and one which the local council has just spent £350,000. It is hoped that new business from the science park will boost the uni-

versity's income through profits accruing to the industrial centre.

The University College of Swansea in Wales is also hoping to set up a science park with local government support next year. It has won the council's support to develop a 20-acre site inside a public recreation area adjoining the university campus.

Similar schemes at the universities of Bristol and Southampton, however, have done less well. The University of Bristol has apparently abandoned its plan for a science park for lack of capital and demand from local industry. And the University of Southampton's project is held up after a refusal of planning permission.

Judy Redfearn

British academic business

NRDC prizes

The National Research Development Corporation, now united with the National Enterprise Board in the British Technology Group, hopes to develop the business sense of British academics by awarding five prizes worth £20,000 each for promising schemes to exploit research results. The prizes will be awarded on a regional basis, the overall winner receiving an extra £30,000. The corporation is also promising to consider favourably investing up to £250,000 in each of the winning proposals.

The aim is to encourage more academics to set up companies to exploit their inventions. According to the corporation, many academics have expressed an interest in doing this, although the more usual way of exploiting research results is for the corporation to take out patents which it then tries to license to industry. Some academics have severely criticized that method, claiming that the corporation is too slow and cautious. The new development suggests that the corporation is prepared to call the academics' bluff.

The corporation has always said that it lacks sufficient good ideas from academics. Nevertheless, to win a prize, academics will still have to convince the corporation of the commercial potential of their plans. Applications for the awards are invited by the end of February 1982 from people who have held postdoctoral or postgraduate studentships or a staff post in an academic institution since January 1979 and who have since either set up or are in the process of setting up a company to exploit an invention.

Applications should include a business plan giving a full description of the product to be manufactured and a marketing strategy. Entrants will be assessed on the probability of success of the business, the technical merits of the product (which must be based on novel technology) and the quality of the business plan.

The corporation is undecided about whether to make the awards in subsequent years, preferring first to assess the quality of next year's applications. **Judy Redfearn**

Swedish universities

More means fewer

Stockholm

Swedish universities are to press the government for an increase in the allocation of students by 10–15 per cent over the next two to three years, to cope with a population bulge among 16–18 year olds — but they believe they can handle the bulge with only a 1 per cent real increase in budget if staff or salaries are cut. These are the current proposals of the Universities och Högskoleämbetet (UHÄ), the national board of universities and colleges which negotiates with the Swedish government.

The proposals are radical in Swedish terms because university grants have been falling by 2 per cent a year for the past three years. Research councils (whose funds, as in most countries, are accounted for separately) have asked for a 4–5 per cent rise, but this is comparable with recent increases (3–4 per cent a year for the past two years) and is in line with a two-year-old commitment by the minister of education to increase science spending by Sw.Kr.70 million (£7 million) per year for three years. Total Swedish research and development spending is relatively low among Western countries, by comparison with gross domestic product, and most political parties in Sweden support an increase in government research spending.

The big target for savings, however, may be the university lecturers themselves. Staff costs account for 80 per cent of the UHÄ budget, and it will be possible to accommodate an increasing number of students within a nearly constant budget only by making savings on salaries. UHÄ has "no policy yet" on whether the cuts will come through redundancies, reduced replacement of retiring staff or pay cuts. Parliament must take a decision on the budget in January.

Robert Walgate

Tropical medicine

Out in the cold

The two principal British centres of education in tropical medicine, in Liverpool and London, last week published a mild but reasoned protest at the damage done by government policy. The two institutions hope to persuade the British government to relax its policy on overseas students' fees on the grounds that if overseas aid as a whole is to be reduced by 15 per cent over the next two years, the government might logically spend more on training in tropical medicine.

The argument by the two schools, the London School of Hygiene and Tropical Medicine and the Liverpool School of Tropical Medicine, appears discreetly as a supplement to *Transactions of the Royal Society of Tropical Medicine* under the title "The present state of tropical medicine in the United Kingdom". Both the London

and Liverpool schools have been hit hard by the increases in fees paid by overseas students, which were increased by government decree in the academic year beginning October 1980. Fees now amount to about £5,000 a year for courses involving clinical instruction, compared with £1,230 in earlier years. Although both schools maintained the numbers of students from overseas in the first year of the new regime — at the London school over 70 per cent of full-time students came from abroad — they are anxious about the effects in the second (and present) academic year, and in any case, they argue, the increased fees do not fully compensate for reduced support from the University Grants Committee.

The Liverpool School of Tropical Medicine, with support from the Wellcome Foundation, produces this 22-page booklet for general practitioners. The value of having such expertise in this country is one of the main planks of the case for the London and Liverpool schools.



The London school has also suffered from the way in which the Court of the University of London has distributed its grant from public funds. In 1979–80, its income of £2.78 million was made up of a 44 per cent subvention from the University Grants Committee (paid through the University of London), 40 per cent from research grants, 6 per cent from students' fees and a similar amount from payments for "services rendered".

The contributors to the society's report, including the society's president Dr A.J. Duggan, of the Wellcome Museum of Medical Science, and Professor D.J. Bradley, director of the Ross Institute of Tropical Hygiene, concentrate on two main arguments. First, the value to developing countries of work done in the United Kingdom on tropical diseases; and second the benefits to the United Kingdom in the form of indigenous expertise of value to the medical profession faced with increasing risks of "tropical" infections contracted by travellers.

The British government recently published its response to the Brandt Commission proposals on Third World development in readiness for the Mexico summit at the end of the month, in which it defends its intention to reduce overseas aid. The Royal Society of Tropical Medicine and Hygiene is putting forward the Liverpool and London schools as cost-effective institutions for helping developing countries, claiming that this is not a one-way process since much of the research in this country on tropical disease is funded by the World Bank and World Health Organization (WHO).

Charles Wenz

Nuclear Bangladesh

Umbrella agreement

Dacca

Bangladesh and the United States have entered into an umbrella agreement for cooperation on the peaceful uses of nuclear energy. The agreement also provides for exchange of knowledge, expertise and equipment.

The United States has agreed to export a 3 megawatt Triga Mark-II research reactor. This will be installed at Savar, about 25 miles north of Dacca and is expected to go into operation in the first half of 1983. It will be purchased by Bangladesh Atomic Energy Commission at a cost of Taka 500 million or approximately US\$26 million on a cash payment basis; an advance of Taka 22.0 million has already been paid to the manufacturer (General Atomic Company). The agreement, for an initial period of ten years which may be extended with the approval of both governments, covers the design, development, construction, operation, maintenance and use of reactors, nuclear fuel disposal, health and safety measures, production and use of radioisotopes in medicine, agriculture and industry. It also includes the exploration and development of nuclear minerals in Bangladesh.

At the signing of the agreements the United States Ambassador to Bangladesh Mrs Jane Abell Coon, recalled that the United States welcomed the accession of Bangladesh in 1979 to the international treaty on the non-proliferation of nuclear weapons.

M. Kabir

Nuclear Yugoslavia

Balkans balkanized

Yugoslavia's first nuclear power station, at Krsko on the Croatian-Slovenian frontier, has at last been completed. A self-sustaining reaction was achieved on 11 September, and low-power tests are now in progress.

Public concern about reactor safety, has presumably been allayed by legislation passed in August dealing with all aspects of nuclear power plant operation. Recent restrictions on power consumption have also done much to swing local opinion back in favour of the Krsko reactor.

Completion of the station, which was built in cooperation with Westinghouse and cost some \$600 million, was more than three years behind schedule, a delay usually attributed to Yugoslavia's lack of transport and construction infrastructure. The Federal Premier, Veselin Djuranovic, however, has blamed construction schedules which, he has said, must from now on be realistically drawn up and properly adhered to. Out of ten power stations scheduled for commissioning in 1981, he said, only one was actually ready.

Loading up for Brazilian power

Rio de Janeiro

Angra I, the first Brazilian nuclear plant — a standard 620 megawatt Westinghouse reactor — has just started loading its 50 tons of enriched uranium rods, some five years behind schedule. At the same time, the president of FURNAS, the electrical company of Rio de Janeiro, has announced a forthcoming increase in electricity rates to "dilute" the \$1,300 million cost. When started, this project was due to cost \$300 per kilowatt; each installed kilowatt has so far cost the Brazilians more than \$2,000.

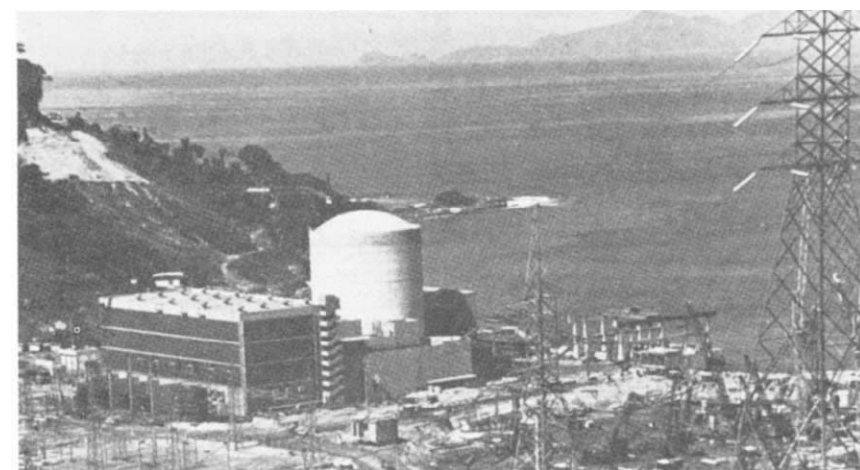
The unit will reach full power by the end of 1981. Westinghouse's personnel will remain on site for one more year — the extent of the guarantee. After that, operations will be suspended for a month to permit partial refuelling with fuel imported from the United States.

The importation of enriched uranium from the United States continues to create diplomatic squabbles between the American government, attempting to impose strict safeguards, and Brazilian

government still refusing to sign the Non-Proliferation Treaty.

So far, though, there is no disposal site for the spent reactor fuel, public protests having forced the abandonment of the initial choice — a vegetable growing valley above Rio de Janeiro. As a temporary measure the spent fuel rods will be stored in the reactor building itself. Low radioactivity waste will probably be stored on an island off Angra's shore.

The other Brazilian nuclear plants, to be built as part of the German-Brazilian contract (see *Nature* 284, 656; 1980), are still a long way off. The 1,200 megawatt Angra II, situated on the same beach as Angra I (left) loaded up, Angra II (right) still waiting for its concrete floor slab to be poured on top of the 300 cement columns built through the sand to reach rock bottom. Its cost, however, keeps rocketing, having already reached \$700 million merely for its foundations; the officially anticipated final cost is \$2,700 per kilowatt delivered. **Maurice Bazin**



Angra I (left) loaded, Angra II (right) waiting

The Premier was visiting the Djerdap (Iron Gates) hydroelectric power station spanning the Danube and built jointly with Romania. Djerdap-1 was commissioned in 1971; Djerdap-2, some 80 km downstream, is still being built and should be commissioned in late 1983 or early 1984. The delays plus the economic recession, which has reduced the country's capacity to import oil and coal, have brought the country's power situation to a point which power engineers at Djerdap described to the Premier as "very critical".

A complication is that Yugoslavia's power industry is organized independently by each republic. Of the three "developed" republics, Slovenia is fairly well-placed, having its own uranium, and the chance, if currency permits, of importing electricity from Austria. Croatia, which already has a share in Krsko, is planning at least two more nuclear power stations.

In Serbia, however, the hydroelectric potential is already 75 per cent exploited.

Coal reserves in Serbia proper are running out (although the would-be-breakaway Autonomous Province of Kosovo still has significant amounts of coal). According to Mileta Jasic, head of the management committee of the republic's Community of Interest for the Power Economy, the "permanent solution" lies in nuclear power (the first nuclear station, he said, could be started in 1990) together with joint undertakings with other republics.

Such cooperation is essential as this republic-based organization can lead to discrepancies. In particular, Yugoslavia's drive to save electricity is being implemented in a rather piecemeal manner. In Croatia, television programmes are closing down early, in Montenegro there has been a flat reduction of 10 per cent to all consumers, while Serbia has introduced a weekly quota — anyone exceeding it will have his next quota docked by an equivalent amount.

Vera Rich

CORRESPONDENCE

Unsöld on Einstein

SIR — We find the statements regarding Einstein made by Professor Unsöld (see *Nature* 16 April, p.535) outrageous and would like to express our strong disagreement. This is particularly important to us now since, in a later letter to *Nature* (4 June, p.374), Professor Unsöld implies that the physics department of the University of Marburg might essentially share his opinion because nobody took issue during the discussion following his colloquium in April 1980.

At the end of his colloquium, Professor Unsöld made various remarks regarding the responsibility of scientists for the application of their discoveries and, in this context, mentioned such names as Einstein and Haber. Some members of the audience had the impression that in so doing he was attempting to compare the responsibility of Hitler and the Nazi leaders for the holocaust with the responsibility of Einstein for the atomic bomb.

In fact, this point was not further discussed after his talk, and one can easily guess why. The title of the talk was "Evolution of cosmic, biological and mental structures", and the remarks concerning Einstein and his relation to the atomic bomb came at the end of a very long talk when Professor Unsöld had by far exceeded the time allotted. The tired audience probably considered these remarks a slip of the tongue by the speaker. However, Professor Unsöld in *Physikalische Blätter* greatly elaborates on his opinion on the responsibility of Einstein. Therefore, we find it imperative to protest at his views.

In his contribution to *Physikalische Blätter*, Professor Unsöld attempts to remove Einstein from the allegedly unwarranted pedestal on which he was placed by some speakers at the meetings during the Einstein centenary. He also tries to revise opinion on the scientific merits of Einstein. It is, of course, quite possible that a judgement on Einstein's achievements in physics might lead to conflicting views among physicists. We dare say, however, that Professor Unsöld's evaluation of the great discoveries of Einstein is unacceptable. However, this is not the point in question.

Professor Unsöld sought to prove his theory that an ever wider gap appears between the

intellectual capacities of physicists to obtain far-reaching knowledge and their moral qualities, which would guarantee that they use their knowledge in a responsible way. He makes the physicists solely responsible.

Einstein was the example chosen to prove this theory. We believe that this theory is disputable in principle. Responsibility for the achievements of science does not rest only with the physicists, but concerns the entire human community. Professor Unsöld attempts to present Einstein as a person of dubious morals and to show that it was quite consistent with Einstein's nature that he should make the "criminal" (in Professor Unsöld's view) decision to write a letter to Roosevelt pointing out the possibility of constructing the atomic bomb. The subsequent use of this bomb at Hiroshima and Nagasaki appears to him to be the work of Einstein. He then compares the "criminal" behaviour of Einstein with the "highly moral" behaviour of German physicists who, despite their sufferings during the Hitler regime, managed to "do some teaching and to safeguard the libraries".

We find it unthinkable that, thirty-five years after the greatest crime in history, a German physicist dares to accuse a man who had to flee Germany to save his life, of criminal behaviour, when indeed he was obviously attempting to help mankind defend itself against the perpetrators of this very crime.

As members of the physics faculty of the University of Marburg, we consider Professor Unsöld's remarks totally unacceptable, and do not wish to condone them by remaining silent. We believe that it is the duty of every German citizen to remember the recent historical events. The written and oral statements of Professor Unsöld certainly do not demonstrate this. Moreover, the seeming acceptance of his statement by some people suggests that some German physicists are no longer conscious of this shameful past. We hope that this "discussion" about Einstein will contribute to an improvement in this direction.

HOLGER NEUMANN, OLAF MEISHEIMER,
WOLFGANG ADAMCZAK, REINHARD ECKHORN,
JÜRGEN ALTMANN, WOLFGANG BAYER,
RALF BECKMANN, NORBERT STELTE,
REINHARD BRANDT, LUDWIG SCHWEITZER,
GUSTAV SAUER, PETER THOMAS
Philipps-University of Marburg, FRG

on its own, I agree that political statements such as those recently published in Nabi's name should be evaluated with knowledge of their author. Indeed, Nabi's consultants are politically diverse. While I am not a sociobiologist, my political opinions do not resemble those of Levins and Lewontin; neither did MacArthur's. However, this did not affect our collaborations.

Our consultation with Nabi was scientific, intended to further an analytic and unified approach to evolutionary biology, an approach which was then very unfashionable. Nabi's book, however, was only partly written when circumstances caused its abortion.

Nabi has survived, mostly, past his 71st birthday (on the same date as Mendel's), and his service in Czechoslovakia for the US Office of Strategic Services during World War II, for which he received US citizenship, was perhaps more dangerous than the public eye.

LEIGH M. VAN VALEN
University of Chicago, USA

Councils of dispare

SIR — During the past 12 months or so I have submitted about a dozen papers to various journals and about a dozen grant applications to various grant awarding bodies. I have also refereed about a dozen papers and a rather smaller number of grant applications.

I am sorry to say that not all my own submissions have been 100 per cent successful on their first attempt. Nevertheless, from the journals I usually receive copies of the referees' reports (often containing helpful suggestions) together with an overall assessment from the editorial office. From the research councils, however, almost no information at all is produced about reasons for rejection.

The curious thing is that as a referee I spend about as much time refereeing a grant application as a paper and submit reports of roughly comparable length. I know that the secretaries who service the research council committees have to prepare minutes. Why is one group prepared to be so constructive whereas the other is so negative? I do hope it is nothing to do with the fact that journals exist at least partly to make a profit (either for shareholders or for the members of some scientific society) whereas the research councils lack such an aim!

ALAN D.B. MALCOLM

*Biochemistry Department,
St Mary's Hospital Medical School,
London, UK*

Book learning

SIR — The recent letters from Andrew Brooks (*Nature* 7 May, p.7) and Robert Campbell (*Nature* 28 May, p.278) about the problems of retrieving information do not touch on a much more serious problem — the need for librarians with some subject expertise.

Expecting students and researchers to learn about as well as maintain currency in both the intricacies of data base searching and the wide variety of printed data sources is simply unrealistic.

Asking for help is obviously *de rigueur* for efficient use of libraries. Are libraries meeting this challenge by recruiting staff members with some subject expertise, especially in the sciences?

DANA L. ROTH
*Robert A. Millikan Memorial Library,
California Institute of Technology,
Pasadena, USA*

Stirling service

SIR — The general features of the cuts recently imposed on the university system by the University Grants Committee are now widely known. What is less well known is how arbitrary and inequitable some of those cuts appear to be. At the University of Stirling, the largest cuts (around 35 per cent) are to be in the sciences and although the physical sciences are to be given some priority, it is beyond question that our own department will suffer in terms of reduced resources. We would like to bring to the attention of the scientific community the inequity of what is proposed.

The Chemistry Department at Stirling University is one of the smallest university chemistry departments in Britain, and the

Nabi — A life

SIR — I would like to make some corrections and additions regarding Isidore Nabi, now that his cat has partly emerged from its bag (*Nature* 3 September, p.2).

The committee called Nabi was formed in the early 1960s, with a programme analogous to, but much less ambitious than, that of the French mathematician Nicholas Bourbaki. Nabi's initial consultants were Richard Levins (not Lester), then at the University of Puerto Rico, Richard Lewontin, then at the University of Rochester, the late Robert MacArthur, then at the University of Pennsylvania, and myself, then at the American Museum of Natural History. Three of us later moved to the University of Chicago, which had no role initially. I believe that Edward O. Wilson, then as now at Harvard University, became peripherally associated for a while.

While scientific work can ordinarily stand

Continued on page 496

Careers of non-medical graduates in British medical research

Judy Sadler, Ruth Porter & David Evered*

THE major functions of a university department in a medical faculty are research, teaching and, in the case of clinical departments, patient care. It is not possible to quantify the relative importance of these various functions, but it is generally agreed that they are inter-dependent and that the creation of an environment in which these activities can thrive plays a major role in improving the quality of medical care. The contribution which the basic biological sciences have made to medical research has increased substantially in the past two decades — and increasing numbers of non-medical graduates are engaged in medical research both within and without medical faculties^{1,2}. The importance of these contributions has been emphasized by various commentators^{3,4}.

However, non-medical graduates in medical research and others have recently expressed their concern that no adequate career structure exists for those without a medical degree⁵⁻¹⁰. Career opportunities are available to this group of scientists in Medical Research Council institutes and units¹¹, some privately funded research institutes and in non-clinical biomedical departments in the universities. But it has been argued that there are insufficient tenured posts to enable full-time research workers in the medical sciences to develop their ideas and skills to the full^{12,13}. The controversy over the proper balance between short-term and long-term support for non-medical graduates in medical research has continued in the columns of many journals during the past three years. This debate has been taking place despite a remarkable lack of data on the distribution, nature of employment, qualifications, age, sex and sources of support of this group. This study was designed to remedy this deficiency and provide the necessary basic data for informed discussion.

Survey method

The study was designed to collect data on all non-medical scientists engaged in medical research in academic and non-profit making institutions. A questionnaire was designed and validated in a pilot survey. The questionnaire in its definitive form was sent personally addressed to the head of each department or unit listed in the panel on the right with an explanatory letter. Reminders were sent 3 weeks and 6

weeks after despatch of the original questionnaire to those failing to respond.

The questionnaire was designed to elicit the following information:

- (1) Number of graduates engaged in medical research (medical and non-medical graduates).
- (2) The status of employment of non-medical scientists (permanent

received from 798 university departments, 75 MRC and other research institutes and units, 17 from Public Health Laboratory Service units, 12 from Blood Transfusion Service laboratories and 4 from other research units.

Medical research was carried out in 752 (83 per cent) of these departments and units and non-medical graduates were engaged

A survey to establish the numbers, status, professional qualifications and age of non-medical science graduates in medical research has been carried out by postal questionnaire. It shows that 31 per cent of all non-medical scientists hold permanent academic posts — but 56 per cent of those with a PhD and 72 per cent of all those aged 35 or over have permanent positions; 42 per cent of non-medical scientists are purely grant supported. Amongst scientists dependent on short-term grant support, 14.1 per cent had received support of this nature for 7 or more years and 4.7 per cent for 10 or more years.

academic, limited-tenure: academic, permanent technician, limited-term technician or short-term grant support only).

(3) Academic qualifications (that is, PhD, other higher degree or first degree only) broken down by employment category.

(4) Ages (less than 25, 25–34, 35 or over) broken down by employment category.

(5) Duration of grant support for those with non-tenured positions.

(6) Sources of grant support.

The totals and numbers of males and females in each category were noted. All the data were also analysed by discipline of department, the nature of employing institutions and geographical area.

The survey was only concerned with non-medical graduates apart from the single question intended to identify the total number of medical graduates in research. Respondents were encouraged to record all non-medical graduates engaged in clinically orientated and basic biomedical research — either full-time or for the majority of their time. Graduates employed in an administrative support role were specifically excluded.

Results

A total of 1,146 questionnaires were sent out and 942 were returned (82 per cent) and of these 36 showed internal inconsistencies which made them unsuitable for analysis. The analysis, therefore, relates to the remaining 906 questionnaires (79 per cent of the original sample). Returns were

in research in 680 departments and units (75 per cent of the total and 90 per cent of those engaged in research). A total of 2,855 medical graduates (of whom 2,458 (86 per cent) were male) and 7,950 non-medical graduates (of whom 5,155 (65 per cent) were male) were engaged in medical research. The male:female ratio for medical graduates was 6.2:1 and for non-medical graduates 1.9:1.

The employment status of the non-medical graduates in the study is shown in

The survey population

(1) All university departments in medical faculties in the United Kingdom.

(2) All university departments of biochemistry, biology, genetics, immunology, microbiology, psychology and zoology in science faculties in the United Kingdom.

(3) All Medical Research Council institutes and units.

(4) All institutes funded by medical research charities.

(5) Central Public Health Laboratories, reference and special laboratories and units, and regional and area laboratories.

(6) Regional Blood Transfusion Service centres.

● The address and name of the head of each unit were taken from *Medical Directory, 1979* (Churchill Livingstone, Edinburgh), *Research in British Universities, Polytechnics and Colleges 1979/80* (British Library), *European Research Index, 4th Edn 1977* (Francis Hodgson, Guernsey), *MRC Handbook 1978/79*, *Handbook of British Medical Research Charities 1979*, *The Hospital and Health Service Year Book 1979* (Institute of Health Service Administrators), and *Public Health Service Directory 1977* (London Public Health Laboratory Service).

*The Ciba Foundation, 41 Portland Place, London W1N 4BN, UK

Table 1 Employment status of non-medical graduates in medical research

EMPLOYMENT STATUS	MALE		FEMALE		ALL		M:F RATIO
	Total	%	Total	%	Total	%	
Permanent academic post	2,009	39.0	423	15.2	2,432	30.6	4.7:1
Limited-tenure academic post	717	13.9	410	14.7	1,127	14.2	1.7:1
Permanent technician post	395	7.7	365	13.1	760	9.6	1.1:1
Limited-term technician post	125	2.4	194	7.0	319	4.0	0.6:1
Grant support	1,909	37.0	1,393	50.0	3,302	41.6	1.4:1

Table 1. It will be noted that 31 per cent have permanent academic posts (M:F 4.7:1), a further 14 per cent have limited-tenure academic posts (M:F 1.7:1) and 42 per cent are purely grant supported (M:F 1.4:1). A significant number of graduates (13.6 per cent of the total) are employed as technicians and over half of these are female. The population was further broken down with respect to their qualifications and these data are shown in Table 2. Forty-five per cent of non-medical graduates in medical research have a PhD and the majority of these are male (53 per cent of the males compared with 28 per cent of females). Eighty per cent of those with a permanent academic post had a PhD (and a further 8 per cent some other higher degree) — and there was some difference between the sexes since 90 per cent of males but only 77 per cent of females with permanent positions had higher degrees. These differences, however, only partly reflect the fact that a much higher proportion of males hold a PhD and 62 per cent of the males but only 35 per cent of females with a PhD have a permanent post. Conversely, higher proportions of females with a PhD have limited-tenure academic posts (25 per cent) or are purely reliant upon grant support (37 per cent) by comparison with males holding a PhD (18 per cent and 19 per cent respectively). A very small number of graduates with a PhD are employed as technicians — 56 in total of whom only 10 are in limited-term employment.

The population was analysed with

respect to age (Table 3) and each subject was assigned to one of three age groups. The largest group (45 per cent of the total) was between the ages of 25 and 34, and 29 per cent of the population was aged 35 and over. The males were generally older than the females with 34 per cent being in the oldest age category and 23 per cent in the youngest — compared with 19 per cent and 32 per cent respectively for the females. Only 47 per cent of females aged 35 or over have permanent posts compared with 79 per cent of males — and conversely 31 per cent of females and only 10 per cent of males in the same age group are purely dependent upon grant support.

Analysis of the population by discipline revealed wide variations in the numbers of non-medical (and medical) graduates engaged in research (see Table 4) the largest number being in biochemistry and the smallest in radiology. Correction of these figures on a discipline by discipline basis indicates that there are 10,155 non-medical graduates engaged in medical research in the United Kingdom in the disciplines and institutions surveyed. The patterns of employment vary considerably from discipline to discipline. Permanent posts are held by 50 per cent or more of the non-medical graduates in occupational medicine (76 per cent), medical physics (56 per cent) and otorhinolaryngology (50 per cent) — and by 40 per cent of the non-medical graduates in anatomy, haematology and forensic medicine. At the other end of the scale permanent posts are available for less than 10 per cent of the

non-medical graduates in research in radiology, venereology and geriatric medicine — it must be added that the total number of non-medical graduates in these disciplines is very small. It was noted that four disciplines relied on grant support for more than 60 per cent of their non-medical graduates (neurology and neurosurgery, dermatology, immunology, and cardiothoracic medicine and surgery) and that four disciplines had a large proportion of non-medical graduates in technician posts (radiology 50 per cent, venereology 46 per cent, medicine 33 per cent and surgery 30 per cent).

The frequency of different qualifications also varied widely between disciplines. Fifty per cent or more of the science graduates in radiotherapy, pathology, physiology, medical physics, anatomy and biochemistry held a PhD. A large proportion of graduates with a first degree only were seen in radiology (83 per cent), general practice (75 per cent), geriatric medicine (65 per cent), cardiothoracic medicine and surgery (63 per cent) and venereology (62 per cent). Some differences in age distribution were seen between disciplines with a relatively high proportion of non-medical graduates aged 35 or over in radiology, radiotherapy, child health and infectious diseases and tropical medicine.

Institution type

The survey results were also analysed with respect to the nature of the employing institution. The research institutes and university departments had similar proportions of their staff in permanent academic posts. The institutes also supported a relatively large number of limited-tenure established posts and were considerably less dependent upon short-term grant support than university departments. The Public Health and Blood Transfusion Laboratories had a high proportion of permanent posts reflecting their heavy service commitments. There were no major differences between the types of organization with respect to the qualification of the non-medical graduates although a slightly higher proportion of those employed in research institutes or units had a PhD or other higher qualification than did those in the university population and the lowest proportion with a higher qualification was seen in public health laboratories and blood transfusion services. The age distribution in the different organizations was similar.

Grant support

The study was extended with respect to those graduates dependent upon grant support. The survey identified 4,748 non-medical graduates with limited-tenure employment and data on the duration and nature of grant support were available for 4,155 (88 per cent) of this group (a proportion of the remainder held posts of limited-tenure which were not dependent

Table 2 Qualifications by status of employment (% in each employment category)

	MALES			FEMALES			ALL		
	PhD	Other higher degree	First degree only	PhD	Other higher degree	First degree only	PhD	Other higher degree	First degree only
Permanent academic post	1,681 (83.2%)	148 (7.3%)	191 (9.5%)	277 (65.3%)	50 (11.8%)	97 (22.9%)	1,958 (80.1%)	198 (8.1%)	288 (11.8%)
Limited-tenure academic post	483 (67.9%)	69 (9.7%)	159 (22.4%)	199 (47.7%)	62 (14.9%)	156 (37.4%)	682 (60.5%)	131 (11.6%)	315 (28.9%)
Permanent technician post	30 (7.6%)	63 (16.0%)	301 (76.4%)	16 (4.4%)	42 (11.5%)	307 (84.1%)	46 (6.1%)	105 (13.8%)	608 (80.1%)
Limited-term technician post	5 (4.0%)	11 (8.8%)	109 (87.2%)	5 (2.6%)	9 (4.7%)	178 (92.7%)	10 (3.2%)	20 (6.3%)	287 (90.5%)
Grant support	515 (28.0%)	210 (11.4%)	1,116 (60.6%)	293 (21.2%)	157 (11.4%)	929 (67.4%)	808 (25.1%)	367 (11.4%)	2,045 (63.5%)
TOTALS	2,714 (53.4%)	501 (9.8%)	1,876 (36.8%)	790 (28.4%)	320 (11.5%)	1,667 (60.1%)	3,504 (44.5%)	821 (10.4%)	3,543 (45.1%)

upon short-term grant support). The duration of grant support for these graduates is shown in Table 5. The duration of grant support is similar for both sexes — 86 per cent of males and females have been receiving grant support for less than 6 years and 14 per cent for 7 years or more. The source of the present grant was the MRC for 34 per cent of these graduates, other research councils for 12 per cent, health service funds for 9 per cent, medical research charities for 21 per cent and other sources for 24 per cent. The latter category included overseas sources, pharmaceutical companies and government departments other than the Department of Health and Social Security and the Scottish Home and Health Department.

Discussion

The survey was carried out in the spring and early summer of 1980 and provides data on the numbers, distribution and characteristics of graduates engaged largely or wholly in medically orientated research at that time. It is purely a cross-sectional survey and thus suffers from the inherent disadvantages of all prevalence studies. The particular advantages of this survey are that a whole population was surveyed (rather than just a sample) and the response rate was high for a postal survey. It is clear that non-medical scientists constitute a substantial proportion (74 per cent) of this population — and that there are probably approximately 10,000 non-medical scientists in research in the United Kingdom in the institutions surveyed. This latter figure allows for the non-responders and is likely to be of the right order of magnitude since the response rate of those disciplines with large numbers of graduates in research was very similar to those disciplines with only a minor commitment to research. It is clear that this survey underestimates the total number of graduates in biomedical research since it does not include those in industry (particularly the pharmaceutical industry and those companies with an interest in biotechnology) and it also excluded research workers in some other university departments or research institutes (for example in the agricultural sciences) whose basic biological work may have particular relevance for the medical sciences. This study also does not include those scientists who are primarily employed to provide laboratory services, who may make occasional, but important, contributions to the pool of medical knowledge.

Sex difference

The employment status of the non-medical scientists was examined in detail, and related to their age and qualifications. There were some differences between the sexes in that a larger proportion of males had a higher degree and they were in general older than the females engaged in research. Even when these factors were

Table 3 Age distribution by status of employment (% in each employment category)

	MALES			FEMALES			ALL		
	< 25	25–34	35 +	< 25	25–34	35 +	< 25	25–34	35 +
Permanent	38	600	1,365	17	154	251	55	754	1,616
academic post	(1.9%)	(30.0%)	(68.1%)	(4.0%)	(36.5%)	(59.5%)	(2.3%)	(31.1%)	(66.6%)
Limited-tenure	104	512	94	98	246	61	202	758	155
academic post	(14.6%)	(72.1%)	(13.3%)	(24.2%)	(60.7%)	(15.1%)	(18.1%)	(68.0%)	(13.9%)
Permanent	89	218	85	94	228	42	183	446	127
technician post	(22.7%)	(55.6%)	(21.7%)	(25.8%)	(62.6%)	(11.6%)	(24.2%)	(59.0%)	(16.8%)
Limited-term	58	56	11	82	91	14	140	147	25
technician post	(46.4%)	(44.8%)	(8.8%)	(43.9%)	(48.7%)	(7.4%)	(44.9%)	(47.1%)	(8.0%)
Grant support	871	844	170	588	623	166	1,459	1,467	336
	(46.2%)	(44.8%)	(9.0%)	(42.7%)	(45.2%)	(12.1%)	(44.7%)	(45.0%)	(10.3%)
TOTALS	1,160	2,230	1,725	879	1,342	534	2,039	3,572	2,259
	(22.7%)	(43.6%)	(33.7%)	(31.9%)	(48.7%)	(19.4%)	(25.9%)	(45.4%)	(28.7%)

Table 4 Distribution of non-medical and medical graduates by discipline

Discipline	No. of institutions responding	Non-medical graduates		Medical graduates	
		No.	Mean	No.	Mean
Anaesthetics	19	30	1.6	66	3.5
Anatomy	25	207	8.3	132	5.3
Biochemistry	49	1,130	38.3	28	0.7
Biology	36	485	13.5	15	0.4
Cancer (oncology) (1)	9	17	1.9	22	2.4
Cardio-thoracic medicine & surgery	10	45	4.5	51	5.1
Chemical pathology (2)	26	407	15.7	104	4.0
Child health	21	55	2.6	98	4.7
Community medicine	31	187	6.0	121	3.9
Dermatology	17	51	3.0	50	2.9
Forensic medicine	10	15	1.5	8	0.8
General practice	12	8	0.7	25	2.1
Genetics	18	218	12.1	23	1.3
Geriatric medicine	8	17	2.1	14	1.8
Haematology (3)	25	114	4.6	71	2.8
Immunology	5	84	16.8	26	5.2
Infectious diseases & tropical medicine	15	104	6.9	28	1.9
Medical physics	9	346	18.2	19	1.0
Medicine (4)	49	560	11.4	467	9.5
Mental health & psychiatry	30	398	13.3	124	4.1
Microbiology (5)	60	399	6.6	130	2.2
Miscellaneous (6)	35	192	5.5	24	0.7
Neurology & neurosurgery	19	38	2.0	53	2.8
Obstetrics & gynaecology	28	121	4.3	133	4.8
Occupational medicine	25	90	3.6	13	0.5
Ophthalmology	19	43	2.3	50	2.6
Orthopaedics	15	49	3.3	49	3.3
Otorhinolaryngology	14	20	1.4	21	1.5
Pathology	44	529	12.0	222	5.0
Physiology	34	704	20.7	180	5.3
Pharmacology	28	482	17.2	63	2.3
Psychology	41	192	4.7	7	0.2
Radiology	18	6	0.3	37	2.1
Radiotherapy	16	97	6.1	29	1.8
Surgery (7)	40	136	3.4	210	5.3
Therapeutics & clinical pharmacology	19	191	10.1	99	5.2
Venereology	12	13	1.1	35	2.9
Zoology	33	275	8.3	8	0.2

(1) Cancer research is also included in biology, pathology and radiotherapy.

(2) Includes clinical biochemistry, clinical chemistry and metabolism.

(3) Includes blood transfusion.

(4) Includes all medical specialties not classified separately.

(5) Includes bacteriology and virology.

(6) Includes statistics, biomedical engineering, medicinal chemistry, etc.

(7) Includes all surgical specialties not classified separately.

taken into account, male graduates were more likely to obtain a permanent position. There are several possible explanations for these differences and this survey cannot with certainty distinguish between these possibilities. However, precisely the same proportion of females and males who were dependent upon grant support had been recipients of grants for seven years or

more, suggesting that the difference overall is due to the fact that a proportion of females leave medical research but return after a few years.

Disciplines

There were some very considerable differences between the various disciplines. A very weak (and statistically not

significant) positive relationship was noted between the mean number of non-medical scientists in each discipline and the proportion who held permanent academic positions. Generally, the more highly qualified non-medical graduates and the highest proportion with permanent posts, were to be found in the sciences basic to medicine and in those disciplines which commonly carried a substantial service laboratory load in addition to their academic commitments. This impression was confirmed by the high proportion of permanent staff in the Public Health Laboratory Service and the Blood Transfusion Service. The purely clinical disciplines and particularly the 'minor' medical specialties generally had the smallest commitment to medical research, the lowest numbers of medical and non-medical graduates engaged in research and very few permanent posts.

Scope

Several general points should be made in relation to this survey. First, it must be recognized that this pool of non-medical scientists is in equilibrium with other populations of biological scientists in universities, industry, other institutes, government and the schools — some of whom will be occupied in research while others will be undertaking other functions

Table 5 Duration of grant support to 4,155 non-medical graduates

DURATION OF SUPPORT	MALES	FEMALES	ALL
Less than 3 years	961 (40.2%)	808 (45.8%)	1,769 (42.6%)
3–6 years	1,092 (45.6%)	707 (40.1%)	1,798 (43.3%)
7–10 years	231 (9.7%)	159 (9.0%)	390 (9.4%)
More than 10 years	109 (4.5%)	89 (5.1%)	198 (4.7%)

(teaching, administration, technical services and so on). The appropriate relative proportions of these various scientific populations will always be debatable — but it is essential to remember that the career opportunities available to the graduate in the biological sciences are not limited to the institutions which were the subject of this survey. Second, it must be emphasized that this survey does no more than describe a population and its characteristics. There are no available methods for determining the 'ideal' balance between long-term and short-term support, or indeed for establishing the optimum level of investment in biomedical research. The controversy relating to the status, career structure and mechanisms of funding of non-medical scientists in medical research will undoubtedly continue. The major objective of this survey was to provide information, which hitherto has been lacking, as the basis for

this debate. The importance of providing this database is underlined by the observation that approximately 10,000 non-medical scientists are engaged in research in the institutions surveyed.

US study

Recently published data¹⁴ from the United States provide a valuable comparison with the present observations, suggesting that there are approximately 7,000 postdoctoral fellows in the United States without permanent posts, compared with about 1,750 in the United Kingdom identified by the present survey. The two are not directly comparable but even so, closely reflect the ratio of the total and graduate populations in the two countries. The US study contains some longitudinal observations and indicates that the input to the 'post doctoral holding pattern' exceeds the output to tenured positions by 11 per cent. The authors of this report highlight the problem of balancing the needs of the individual scientist against the long-term research requirements of the community. It is clear from our survey and from the US study that long-term policies of support for biomedical research should be based upon objective observations of personnel and performance.

We thank Professor A. G. Shaper and members of the Department of Clinical Epidemiology and Social Medicine, Royal Free Hospital, London, particularly Dr S. J. Pocock and Miss L. M. Carpenter, for statistical advice and help in designing the pilot study and questionnaire, and Brigadier K. David Gribbin (Secretary-General of the Cancer Research Campaign) for his comments. We also thank the many heads of departments and institutes who spent a great deal of time filling in the five-page questionnaire.

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The reasons why

BEHIND both the accompanying study and the recent report commissioned by the US National Science Foundation (see above and also *Nature* 11 June) lies a good deal of unrest amongst junior research scientists. The US report, carried out by the National Academy of Sciences (National Research Council) with a steering committee chaired by Dr Lee Grodzins of the Massachusetts Institute of Science and Technology, gives expression to some of the discontent shown by postdoctorals in the United States and lists amongst its conclusions:

- We have found a lack of concern on the part of most universities for the well being of their postdoctorals as an identifiable group.
- Few universities assume any responsibility for shaping or even monitoring the character of the postdoctoral experience or for ensuring its quality.
- A significant fraction — in some fields the majority — of postdoctorals deplore many of the conditions of their appointments.
- Postdoctoral experience contributes little or nothing in terms of subsequent income.
- Women postdoctorals are more likely than their male counterparts to pursue academic careers, but the men entering academic employment are more likely to get faculty appointments and are more

quickly given tenure.

The supply of secure, tenured academic posts no longer matches the numbers of postdoctoral researchers that are required to keep the 'academic research industry' going (if ever it did) and so there will always be a steady stream of postdoctorals failing to find a niche in academia. The US report recommends that individual universities should set up standing committees to review the conditions of postdoctorals, with emphasis on salary (or stipend) levels, status within the university community, availability of career counselling, and subsequent employment of those completing appointments.

The National Science Foundation already sponsors data collection in the fields of research funding, graduate enrolments, doctoral awards, and science and engineering employment. The report calls for the foundation to introduce surveys on the career decisions of young scientists and engineers and to publish a biennial series of reports on the changing patterns and utilization of postdoctorals and other groups of young investigators. Armed with such information the requested standing committees may be in a position to give some useful advice.

Despite the difficulties though, the report concludes that postdoctoral fellowships make an invaluable contribution to research, and remain a valuable preparation for academic life. □

NEWS AND VIEWS

An uncensored page of fossil history

from J.S. Jones

"A history of the world, imperfectly kept, and written in a changing dialect. Of this history we possess the last volume alone . . . Of this volume, only here and there a short chapter has been preserved; and of each page only here and there a few lines." Thus Darwin on the imperfections of the fossil record, and thus his explanation — widely accepted until recently — of the absence from the record of the transitional forms expected on his view of the origin of species as a result of natural selection which acts "solely by accumulating slight, successive, favourable variations, and can produce no great or sudden modification".

In this issue of *Nature* (p.437), Williamson reports a page of the fossil history of the world which has been preserved more or less complete. He has studied a sequence of fossilized freshwater molluscs from the Turkana Basin in East Africa. In a series of fossil beds 400 m thick, which have yielded important human remains, there are preserved millions of shells of at least 19 species of snail from several genera. These deposits have remained more or less undisturbed since their formation, and the order of formation of each bed can be identified by referring to their relationship with accurately dated local geological features. This evolutionary series presents, as Williamson says, an unprecedented opportunity to study patterns of evolutionary change in a complete fossil record.

On first sight, these patterns are quite different from those expected on the theory of gradual evolution insisted upon by Darwin. In the 13 lineages common enough for detailed analysis, long periods of morphological stability are interrupted by fossil beds in which relatively rapid changes in shell shape take place. These newly evolved populations then persist unchanged through thick deposits before, in most cases, becoming extinct and being replaced by fossils resembling the ancestral forms (some of which persist to the present day). The intermediate forms between the ancestral and derived species occupy only a very small proportion of the evolutionary history of each lineage. The periods of transition coincide with each other in the various genera; including — remarkably

enough — *Melanoides*, a taxon which is in its modern guise an obligate parthenogen. This apparent ability of an asexual species to evolve as rapidly as its sexual relatives casts some doubt on the many theories which claim that sexual reproduction increases evolutionary flexibility¹.

There is no doubt that these patterns of stability interrupted by change are real. In this they differ from recent claims that the fossil history of man shows similar patterns; these rest only on the incompleteness of the human fossil record².

Williamson suggests that the evolution of his snails conforms to a model of change in the fossil record which can be traced to the work of Cuvier in the eighteenth century; was later promoted by Goldschmidt³, and has recently been resurrected as the 'punctuated equilibrium' model of Eldredge and Gould⁴. Evolution is seen as an essentially discontinuous process in which long periods of genetic stability are interrupted by 'genetic revolutions' which produce very rapid change, rather than as a system of gradual change leading to the origin of species by natural selection acting on minor differences in fitness among individuals. Darwin's often quoted claim that "*natura non fecit saltum*" is not correct; the absence of intermediate forms in the fossil record is a genuine result of the mechanisms of evolution, rather than an artefact of the accidents of preservation. To quote Williamson, the patterns of change in his fossil snail populations must indicate that "speciation is a *qualitatively* different phenomenon from gradual intra-specific evolutionary change". The darwinian theory of the origin of species, if not wrong, is at least incomplete.

Does this new information on a uniquely complete palaeontological series indeed mean that geneticists must revise their views of how species originate? Here we see an important difference in the way in which those dealing with fossils and those who experiment on living organisms assess the

rate of evolutionary change; because of the differences in the time scales habitually encountered by the two groups, what is an instant of evolutionary time to a palaeontologist may appear almost an infinity to a geneticist.

Williamson shows that the intermediate forms in his fossil series existed for between 5,000 and 50,000 years, periods much shorter than those of evolutionary stability in each lineage. The living relatives of these snails have a generation interval of between six and twelve months⁵⁻⁷, so that the morphological changes between the stable shell forms took on the average perhaps 20,000 generations to complete. To most geneticists, this interval seems more than sufficient to enable gradual changes to lead to morphological evolution as great as that described by Williamson. It is the equivalent of perhaps a thousand years in a *Drosophila* population cage, six thousand years in a mouse selection experiment, or 40,000 years when dealing with domestic animals such as dogs. Darwinian selection of the most conventional kind has often been used to accomplish dramatic genetic changes in morphology much more rapidly than this. In less than fifty generations of artificial selection on a base population it has been possible to produce selected lines of *Drosophila melanogaster* which have abdominal bristle numbers varying from three to eighty-five⁸; of mice whose body weights are as different as 13 g and 32 g⁹, and of corn in which one selected line has an oil content of 15 per cent compared with only one per cent in that selected in the opposite direction¹⁰. In each case these changes have been achieved by gradualistic selection, and it is not necessary to postulate that new evolutionary mechanisms must be involved.

Reproductive isolation, the central element of speciation can also be achieved by simple darwinian selection in a period much shorter than that assessed by Williamson and others as evidence for the inadequacy of darwinian theory. In *Drosophila melanogaster*, for example, gradualistic selection for preferential mating among mutants in a population cage can lead to considerable reproductive isolation within only 18 generations¹¹, and

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it is possible greatly to alter the degree of sexual isolation of *D. pseudoobscura* from its close relative *D. persimilis* by artificial selection in less than 20 generations¹². In the same way, selection of different parts of an originally freely interbreeding *Drosophila* or house fly population for bristle number, climbing ability or the ability to tolerate insecticides can lead to the incidental evolution of considerable reproductive isolation within a few tens of generations¹³⁻¹⁵. Even *Drosophila* populations kept at different temperatures for five years in the laboratory evolve considerable reproductive isolation among themselves¹⁶. These are periods trivial in relation to those interpreted by Williamson and others as 'punctuations' in the fossil record which can only be explained by new evolutionary mechanisms.

The efficacy of gradual evolutionary change in producing genetic subdivision and the reduction of gene flow in natural populations subject to the forces of gradual selection is particularly well seen in plants. Some annual grasses have evolved an ability to grow on mines polluted by

concentrations of copper high enough to kill populations not exposed to this selective agent. The grasses on the mine flower about a week earlier than do the surrounding non-tolerant populations and have an increased ability for self-fertilization. These genetic differences — which have evolved in the one hundred years since the mines were opened — are enough to lead to considerable reproductive isolation between mine populations and their ancestors in the nearby pastures¹⁷.

Once evolutionary change in the fossil record — even in a record as well characterized as that unearthed by Williamson — is placed in the context of the known ability of living organisms to respond to the forces of classical darwinian natural selection, it becomes clear that it is not necessary to invoke evolutionary forces 'qualitatively different' from those emphasized by Darwin. Depending on the time scale to which the investigator is accustomed, one man's punctuated equilibrium may be another's evolutionary gradualism. Williamson describes an extraordinarily complete page in the history of evolution

but its contents do not force us to change our views on the genetic mechanisms of the origin of species. □

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Diversity changes of marine organisms through the Phanerozoic

from A. Hallam

ONE of the most important palaeontological debates of the past decade appears to have been resolved in an article published in this week's *Nature* (see page 435). The issue at stake is whether the diversity of life has increased substantially in the five hundred million years since the Cambrian period or whether it is only imperfections in the fossil record that give an appearance of increasing diversity while the true pattern is of equilibrium or even decrease in diversity.

The two chief protagonists in the debate, David Raup and James Valentine, are now in agreement and have joined forces with two other American palaeontologists, John Sepkoski and Richard Bambach, who have made notable contributions to the subject. Together they demonstrate that there is indeed, in their choice of phrase, a genuine "evolutionary signal" discernable in the fossil record of marine organisms despite possible preservational and taxonomic noise. The agreement is a firm vote of confidence in the reliability of the fossil record even though the conclusion drawn from it is a sort of compromise — diversity has increased since the Cambrian but the pattern is not one of a simple rise.

It is the existence of such massive data compilations as the American *Treatise on Invertebrate Paleontology* and the Geological Society of London's *The Fossil Record* that have allowed palaeontologists to analyse the pattern of diversity change of preservable organisms

from the Cambrian to the present. To obtain reliable estimates of the extent of change through time it is clearly desirable to be able to eliminate possible complications due to preservational bias and the problems inherent in making taxonomic distinctions among fossils.

Originally, Valentine made the claim that marine invertebrate generic and family diversity increased substantially from the early Palaeozoic to the Cenozoic. This was challenged by Raup soon afterwards (*Science* **1977**; 1065, 1972). He argued that the large Mesozoic–Cenozoic increase may be an artefact. The Cenozoic record includes extant taxa, which biases it towards an overestimate, and preservational factors may have produced a major bias in the reverse direction further back in time. He generated a computer simulation model based on an increased probability of destruction of fossils with time, and showed that Valentine's data could be accounted for by postulating diversity rise from the Cambrian to a mid-Palaeozoic peak followed by a drop to an appreciably more modest level which remained stable through the Mesozoic and Cenozoic.

In a later paper (*Paleobiology* **2**; 289, 1976), Raup estimated the number of species described for each of the geological

periods by tabulating new species reported in the *Zoological Record*, and established a strong correlation between apparent species numbers and the present areal distribution of rocks per system. This suggested that perhaps geological systems with more available rock have more species and hence more species are described.

In the analysis published this week, five independent measures of diversity, for different groups of organisms and different taxonomic levels, are shown to be highly inter-correlated, even when the ubiquitous correlation with time is removed. A simple underlying pattern is revealed of a rise from a low value in the Cambrian through the Palaeozoic, followed by a low Triassic value, after which there is a more or less accelerating increase through the Mesozoic and Cenozoic, the last era containing the highest diversity. The sharp drop at the end of the Palaeozoic is clearly the result of the Permian mass extinction. In sharp contrast the end Cretaceous mass extinction has no effect on the general trend. Above the family level the pattern of diversity change is different, due probably to the tendency for morphologically distinct groups to appear rapidly during the early phases of major radiations.

Palaeontologists will no doubt continue to argue about the meaning of diversity-change data, but at least they are now apparently assured that the data reflect the real world. □

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Charles Darwin and earthworms

from Clive A. Edwards

CHARLES DARWIN is most famous for his work on evolution and his controversial book *'The Origin of Species'*. Much less familiar is another book *'The Formation of Vegetable Mould through the Action of Worms'* (Murray) which appeared in 1881, the year before his death, and sold many copies. Correspondence concerning the book, preserved in Cambridge, has been reviewed by Otto Graff (Braunschweig, FRG) and shows that it was the culmination of more than 40 years of observations first inspired by Darwin's uncle Josiah Wedgwood. The book demonstrated for the first time the importance of earthworms in the breakdown of organic matter and in maintaining soil fertility, a conclusion very much at

odds with the opinion of many of Darwin's contemporaries, who believed that earthworms were harmful to plants and produced a considerable literature describing methods of killing them.

The publication of Darwin's book in October 1881 was recently celebrated at an International Centennial Symposium*. Darwin's belief in the beneficial influences of earthworms stated so clearly 100 years ago was strongly supported by many of the contributions.

For example, it was shown that the production of grass in New Zealand, which is extremely important to sheep farming, could be influenced greatly by earthworm activity. New Zealand does not have native lumbricid earthworms, and experimental inoculation of earthworms of the species *Allobophora caliginosa* has greatly increased yields of grass over that in pastures with no earthworms. Work by S. Stockdill (Department of Agriculture,

New Zealand), J. Syers (Massey University) and J. Springett (Ministry of Agriculture and Fisheries, New Zealand) has shown that the well defined, undecomposed mat of organic matter at the base of the grass breaks down when earthworms are introduced and the available nutrients that are released greatly promote grass growth.

Many workers have claimed that when earthworms are inoculated into cultivated soils with poor natural populations, the growth of plants is increased. This role of earthworms has been shown to be much more important when crops are sown into soil after the use of a herbicide and with no mechanical cultivation (direct drilling). Workers at Rothamsted Experimental Station, UK have established that inoculating earthworms into soils that have been direct drilled for long periods, increases considerably the growth of roots and yield of crops. This was shown to be due to the provision of deep channels for root growth, lined with more available mineral nutrients than the surrounding soil. An earthworm management programme for direct drilled soils proposed by Rothamsted workers involves minimal ancillary cultivations, no straw burning, avoidance of harmful pesticides and addition of large doses of organic matter to soil.

The importance of earthworms in land reclamation was discussed by J.F. Curry and D.C.F. Cotton (University College, Dublin). There is a need for land reclamation after industrial activities such as strip mining and land fill, after inundation by the sea and after peat has been harvested from peat bogs. In all these situations, there is a gradual natural invasion by earthworms after the reclamation process begins, and there is little doubt that the worms have an important role in re-establishing soil structure. Earthworm activity can be promoted and reclamation accelerated by inoculation of earthworms, making soil conditions more favourable for them and by adding large amounts of suitable organic matter such as municipal sludge or animal wastes.

Few people consider earthworms to be a marketable commodity; but the large international traffic in earthworms was reviewed by A.D. Tomlin (Canada Agriculture, London, Ontario). He quoted figures that showed the retail value of earthworms exported from Canada to the US for fishing bait in 1980 was as much as 54 million Canadian dollars, and in addition large quantities were exported to

*The International Darwin Centenary Symposium on Earthworm Ecology was held at Grange-over-Sands, Cumbria (August 29 to September 4 1981) and attended by 142 scientists from 28 countries. The proceedings will be published by Chapman and Hall, London and in the journal *'Pedobiologia'*.



100 years ago

MR. DARWIN ON THE WORK OF WORMS

If the world were not already accustomed to the unprecedented fertility of Mr. Darwin's genius, it might well be disposed to marvel at the appearance of yet another work, now added to the magnificent array of those which bear his name. But feelings of wonder at Mr. Darwin's activity have long ago been sated, and most of us have grown to regard his powers of research as belonging to a class *sui generis*, to which the ordinary measures of working capacity do not apply.

One of the most interesting chapters deals with the habit of dragging down leaves, &c., into the burrows; for here the experiments elicited some very remarkable evidence of action which is apparently intelligent.

Mr. Darwin "observed carefully how worms dragged leaves into their burrows whether by their tips or bases or middle parts. It seemed more especially desirable to do this in the case of plants not natives to our country; for although the habit of dragging leaves into their burrows is undoubtedly instinctive with worms, yet instinct could not tell them how to act in the case of leaves about which their progenitors knew nothing. If, moreover, worms acted solely through instinct or an unvarying inherited impulse, they would draw all kinds of leaves into their burrows in the same manner. If they have no such definite instinct, we might expect that chance would determine whether the tip, base or middle was seized. If both these alternatives are excluded, intelligence alone is left; unless the worm in each case first tries many different methods, and follows that alone which proves possible or the most easy; but to act in this manner and to try different methods makes a near approach to intelligence".

A large number of experiments were therefore tried with leaves of various shapes, and both of endemic and exotic species. The results showed unequivocally

that the part of the leaf which the worm seized for the purpose of dragging the whole into the burrow was not a matter of chance, but in an overwhelming majority of cases that part of a leaf was seized by the dragging of which the leaf would offer least resistance to being drawn into the burrow. Thus, for instance, "the basal margin of the blade in many kinds of leaves forms a large angle with the foot-stalk; and if such a leaf were drawn in by the foot-stalk, the basal margin would come abruptly into contact with the ground on each side of the burrow, and would render the drawing in of the leaf very difficult. Nevertheless worms break through their habit of avoiding the foot-stalk, if this part offers them the most convenient means for drawing leaves into their burrows".

To test the hypothesis of chance, elongated triangles were cut out of paper and given to the worms instead of leaves. Here "it might certainly have been expected, supposing that worms seized hold of the triangles by chance, that a considerably larger proportion would have been dragged in by the basal than by the apical part"; while, inasmuch as the latter was in a literal sense the thin end of the wedge, it was the part which intelligent action would be most likely to choose. The results of many experiments with these paper triangles showed that "nearly three times as many were drawn in by the apex as by the base. . . . We may therefore conclude that the manner in which the triangles are drawn into the burrows is not a matter of chance, . . . and we may infer — improbable as is the inference — that worms are able by some means to judge which is the best end by which to draw triangles of paper into their burrows".

On the question of defining such action as intelligent or non-intelligent, Mr. Darwin refers to the criterion "that we can safely infer intelligence only when we see an individual profiting by its own individual experience"; and he adds that "if worms are able to judge, either before or after having drawn an object close to the mouths of their burrows, how best to drag it in, they must acquire some notion of its general shape", and thus guide their actions by the result of individual experience.

Assuredly these observations are most interesting, and it would seem well worth while to try whether, by a series of lessons with similar triangles of paper, an individual worm could be taught to lay hold of the apex in a greater and greater proportional number of cases; if so, there could no longer be any question as to the intelligent nature of the action.

GEORGE G. ROMANES

From *Nature* 24, 13 October, 553, 1881.

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Europe. These earthworms were obtained either by experienced 'pickers', each collecting up to 10,000 *Lumbricus terrestris* per night from golf courses, or by breeding *Eisenia foetida* or *Eudrilus eugeniae* in a wide range of human or organic wastes. The potential of earthworms in degradation of organic wastes and as a source of food and drugs was reviewed by J. Sabine (Waite Institute, Adelaide, Australia) and discussed by contributors from several other countries as

far afield as the US, Japan, Nigeria and the Philippines. Most of these groups have concentrated on the production of a material suitable as a plant growth medium or a soil additive, from a wide range of organic wastes, by the action of earthworms.

There seems little doubt that Darwin's faith in the usefulness of earthworms to man has been fully justified and it seems likely that these humble creatures may prove to be even more valuable during the next decade. □

The biology of oligodendroglia

from a correspondent

OLIGODENDROGLIAL cells have the important role of producing the myelin sheath of axons in the central nervous system. The formation of myelin results in changes that facilitate the conduction of impulses. The study of oligodendroglia and myelin is therefore of considerable importance in neurobiology and has relevance to several human diseases, particularly multiple sclerosis (MS).

At a workshop* held in June, recent biochemical studies of myelin in whole brain or in tissue slices and of oligodendrocytes in mixed cell cultures were discussed. These investigations provide new insights into the development and differentiation of oligodendrocytes, the synthesis of myelin and gene expression of oligodendrocytes in some non-myelinating systems. It appears that, during development, the expression of enzymes responsible for the synthesis of myelin components and the production of these components are coordinated but that many of these events do not occur simultaneously. For example, the activity of cerebroside sulphotransferase (CST), the enzyme that converts cerebroside to sulphatide, rises before that of UDP galactose:ceramide galactosyltransferase (CGalt), the enzyme that catalyses the last step of cerebroside synthesis. This puzzling finding may imply that most of the cerebroside formed before myelination is channeled into sulphatide formation. Both these myelin lipids can be detected before myelin basic protein (MBP) appears. Several other enzymes of lipid synthesis also increase dramatically before and during myelination and then decline. Although the control mechanisms that regulate such enzyme activity are poorly understood, some interdependencies have now been established. For example, the activity of the enzyme glycerol phosphate dehydrogenase is induced specifically in oligodendrocytes by cortisol and the

oligodendrocyte-specific enzyme 2', 3'-cyclic nucleotide 3' phosphodiesterase is stimulated in glioma lines by noradrenaline via a cyclic AMP-dependent mechanism. The activity of CST appears partially to depend on the ratio of cholesterol to lecithin in membranes containing the enzyme; such ratios can be altered experimentally by adding appropriate liposomes *in vitro*.

It has also been possible to localize several enzymes (including CST, CGalt and plasmalogenase) to oligodendrocytes using bulk-isolated enriched cell fractions.

The assembly of myelin components by the cell into myelin membranes is being examined by kinetic studies combined with protein synthesis inhibitors. The two major myelin proteins, MBP and proteolipid protein (PLP), are assembled into myelin at different times after synthesis: the incorporation of PLP into myelin lags behind that of MBP by several minutes and continues for several minutes after protein synthesis has stopped. These data imply that PLP is processed after synthesis and is assembled into a different precursor membrane than MBP. Preliminary results indicate that it is possible to isolate populations of precursor membrane vesicles that represent both different stages in the formation of myelin precursors and separate pathways for carrying different myelin proteins.

After assembly, the myelin constituents turnover quite rapidly and at different rates. Constituents added early in development are removed at a slower rate than those added later ('last in, first out'). Each lipid has its own turnover time but, in general, lipids turnover faster than proteins and some proteins formed early in life are metabolically very stable. The more rapidly metabolized proteins may not be homogeneously distributed within the sheath but are located preferentially in areas peripheral to compacted myelin layers.

Many of these observations on the biochemical behaviour of oligodendroglia *in situ* have been confirmed by investigators studying oligodendroglia in

mixed cell culture systems. *In vitro* preparations show the developmental increases of differentiated properties of oligodendrocytes, such as CST, CGalt, cerebroside and MBP. There is speculation that, whereas Schwann cells will not express differentiated functions in the absence of axons, the expression of these properties in oligodendroglia is partly pre-programmed into the cell and does not require feedback signals from axons for induction of many of its components.

Several other recent *in vivo* and *in vitro* studies provide new insights into the role of cell interactions in myelination. Addition of rat glia from dissociated embryonic spinal cord to cultures of dorsal root ganglia results in proliferation of the ensheathing cells and also in extensive myelination — additional proof that peripheral nervous system axons can promote glial mitosis and differentiation. *In vivo* experiments using mouse chimaeras obtained by aggregation of eight-cell preimplantation embryos have recently permitted the study of interactions between normal and mutant glia throughout primary development. Chimaeras and other techniques such as nerve grafting may also elucidate the interactions between the glial cells themselves and between glia and axons in the abortive remyelination and regeneration that follows various forms of central nervous system injury. Additional benefits will be derived from recent improvements in the preparation of separate cultures of astroglia and oligodendroglia and from the very exciting advances made in the development of specific markers for different types of glia and neurone.

Using immunological techniques, glia and neurones can be identified and purified independently of morphological characteristics. This methodology has been revolutionized by procedures for the derivation of monoclonal antibodies. Purified cell populations should help characterize the molecules involved in myelin induction and in the control of glial cell proliferation.

It is not yet known whether the oligodendrocytes or the myelin sheath itself is the main target in MS. Using organotypic central nervous system cultures and adding myelinotoxic MS sera in the presence of complement, there is a selective destruction of oligodendrocytes *in vitro*. However these observations have not been corroborated in MS lesions displaying ongoing demyelination where oligodendroglia do not appear to be selectively destroyed in the early lesions. Immunocytochemical studies of MS plaques also suggest that the mechanism of myelin breakdown in MS may be immune-ligand-mediated phagocytosis rather than a failure of the oligodendrocytes. Thus, in MS, the mechanisms that lead to the loss of myelin-forming cells and to the limited repair of demyelination remain to be clarified. □

*A multidisciplinary, international group of about 60 investigators gathered in June at Arlie House, Virginia, under the auspices of the National Multiple Sclerosis Society of America to discuss recent advances in the biology and pathology of oligodendroglial cells.

Tracing outbreaks of foot-and-mouth disease

from J.B. Brooksby

FOOT-AND-MOUTH disease is transferred remarkably easily and there is little difficulty in accounting for the infection of animals which have been in contact with the disease at markets or on neighbouring farms. Simple explanations often apply for the spread of the disease over longer distances too: the dispersal of the animals from markets or the movements of infected animals products or foodstuffs may be the cause. Between countries and continents, however, the route is frequently less direct, as in the case of the meat trade from S. America to Europe, and it becomes necessary to establish both the epidemiological facts and the survival characteristics of the virus.

In the past the investigation of such spread had much in common with the plot of a detective novel, showing great ingenuity but less objectivity. Recently, however, technical advances and new epidemiological findings, as illustrated on p479 of this issue of *Nature*, are making possible better judgements both on the pathways and on the identification of the virus involved.

Following the 1967–68 outbreak in the UK the possibility that the virus was being transmitted by air was extensively investigated. Quantitative data on virus excretion, aerial transfer and infection though the respiratory tract were combined with meteorological data to produce models of spread¹ which were then tested retrospectively on field series of outbreaks, in the UK in Hampshire (1967) and Northumberland (1966), between Denmark and Sweden (1966) and from Brittany to the Channel Islands (1974). Cases of transmission across water present fewer complicating factors than over irregular land surfaces and at times the temperature differences at the water-air interface limits the dispersion of the plume of potentially infective air. From the conditions on two dates in March 1981, the model strongly supports the possibility of airborne transmission from Brittany to Jersey and to the Isle of Wight. Most energetic efforts were made to detect other possible sources for the 1981 infection in the Isle of Wight but met with no success whatever.

The second clue to links between outbreaks lies in the identification of the strain of virus. In the 1920s and 30s the method was not of great value as only three serotypes of virus could be distinguished. That two farms were infected with type O virus, for example, could scarcely establish a connection between them. Later, when

serological methods differentiated a continually increasing number of strains, the appearance of foci of infection due to a hitherto unrecognised subtype was stronger evidence for epidemiological links between them. A close parallel existed between this situation and the epidemic spread of new influenza strains in man.

A wave of infection in Western Europe in 1965–67 provides a good example of the importance of sub-types of the virus. At that time cattle were frequently vaccinated in the countries concerned, although sometimes only in the face of a new threat of the disease. The vaccine was generally specific against the sub-type strain O₂, which had been the most important strain in outbreaks in the preceding ten years. When the disease began to appear in vaccinated animals in 1965, it was believed that a totally new strain of virus had appeared.

Laboratory investigation, however, identified the new strain as similar to the earlier sub-type O₁ which had been the most prevalent type O strain until about 1955. Strains of this sub-type seemed to have disappeared from the Western European scene, with the exception of a small outbreak in Belgium in 1963. The spread of the 'new' O₁ strain began in Switzerland (the O Lausanne) and moved through neighbouring countries of Germany, France, Holland and Belgium. The response of the vaccine-producing laboratories was to replace the O₂ strains by the new field strains including the O Lausanne. The reappearance of the O₁ strain is not so surprising as similar strains were present in the Eastern Mediterranean countries, in S. America and possible in Spain in the early 1960s. Minor differences — of possible use in determining origins — did exist among the O₁ group, but efforts were concentrated on those major differences which would affect the success or failure of vaccination of cattle in the field.

Recently a new order of precision has been achieved in the identification of strains. Two biochemical methods have been developed, the fingerprinting of the ribonuclease T oligonucleotides and the electrofocusing of the polypeptides of the virus coat. The use of these methods is graphically illustrated in the paper in this week's *Nature*, which examines the relationship between the strains from the outbreaks in Brittany, Jersey and the Isle of Wight which have already been linked on the basis of airborne spread. The salient feature of the strain comparisons in the paper is that there is absolute identity between strains recovered in Brittany and the Isle of Wight and the strain O Lausanne

which continues to be used as the most effective vaccine strain in several European countries. The authors consider, with justification, that this identity of strains goes beyond coincidence. Their own evidence is that, in biochemical terms, minor changes occur very readily during the spread of viruses in the field. In the 1967–68 outbreak in the UK, for example, although the virus remained serologically an O₁, six biochemically determined sub-strains were found among seven isolates taken during the outbreak. The minor changes observed in the present series, an additional band in VP2 in the Jersey sample and a slight charge-change in P56a in one Isle of Wight sample, if anything strengthen the conclusion that the Brittany, Jersey and Isle of Wight outbreaks of 1981 are related.

There remains the question of the origin of the Brittany outbreak. The paper offers three suggestions but does not express a definite view. However, at a meeting of the European Commission for the Control of Foot-and-Mouth Disease in April 1981 (see ref. 2) it was recognized that as foot-and-mouth disease had been virtually eliminated in Europe but vaccination policies had been maintained, perhaps half the outbreaks which have occurred in the past ten years have arisen from incompletely inactivated vaccine. The inactivation of viral vaccines has always been a difficult matter. In foot-and-mouth disease much attention has been given to the safety-testing of vaccines, Henderson as far back as 1952 (see ref. 3) pointing out the statistical weakness of the most stringent laboratory control, compared with the field use of a batch of vaccine when many millions of doses may be inoculated. An important point in the current situation is that formalin is still in widespread use as an inactivant and that such a vaccine was used in Brittany. This agent has now been superseded by the more progressive producers, including the French group responsible for much of the vaccine used in France.

The matching of identity by biochemical techniques raises many interesting questions. It seems that passage of strains in tissue-culture in the laboratory, as will occur in the production of virus for vaccine, is less likely to change either the genome or the polypeptides than the natural passage of virus in field spread. In the present case the O Lausanne stored for

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3. Henderson, W.M., *J. Hyg. (Camb.)* 50, 195 (1952).

16 years was compared with virus from the field outbreak, which, assuming it arose from the vaccine, had received some serial passage in the laboratory in the production of vaccine seed virus. Serological evidence already exists on changes in foot-and-mouth disease virus under epidemiological

pressures and in laboratory passage in immune serum. The present work, by giving much greater precision to such investigations and revealing the mechanism of change, will be of fundamental importance to the study of the virus. □

Hot and noisy stars

from M.G. Edmunds

AFTER a decade of comparative stagnation, there is renewed interest in the theory and observation of stellar atmospheres. The renaissance is due partly to recent improvements in computing power (the advent and promise of machines like CRAY-1) and partly to observations in new wavelength bands — particularly the UV with the International Explorer satellite and X rays with the Einstein Observatory. In Britain, the Science and Engineering Research Council has financed a collaborative computing project (CCP7), with the aim of encouraging the exchange of stellar atmosphere computing software; a workshop* associated with the project was held in July at Gregynog Hall in mid Wales. Two topics highlighted at the meeting (and which have also figured prominently in recent literature) were hot stars with peculiar helium abundances and velocity fields in stellar atmospheres. The latter is of considerable importance because of its possible relation to chromospheric and coronal activity.

Extensive computational work, described by R. Kudritzki (Kiel University), has allowed the assumption of local thermodynamic equilibrium to be relaxed in calculations of the appearance of emitted spectra, which are compared with observed spectra of the hot stars called 'subdwarfs'. (These stars have considerably smaller radii than normal dwarf stars of the same temperature and probably represent a stage in the life of a star between a bright red giant and a slowly fading white dwarf.) The non-equilibrium calculations have led to a much more reliable determination of the helium abundance in the stars' atmospheres and confirms that a very large variation exists between different stars. There appears to be a gradation in atmospheric composition from helium-poor B-type subdwarfs, with only about one helium atom for every hundred hydrogens, to nearly all helium and almost no hydrogen in the hotter O-type subdwarfs^{1,2}. Related objects are the stars at the centres of planetary nebulae, which

can also show helium deficiency.

Although the high helium abundances can be fairly easily accounted for by normal nuclear processing of hydrogen to helium in a star's interior, which is subsequently mixed to the surface, the helium underabundances are rather harder to explain. The material out of which the stars formed should have contained at least the one atom of helium per ten of hydrogen that resulted from big bang nucleosynthesis, and the most likely explanation of the underabundances is that the atmospheres are untypical of the subdwarf stars as a whole. In fact, the stars will have an overall excess of helium as a result of their previous evolution, but while passing through the subdwarf stage the force of gravity at the surface of these stars is sufficiently strong, and the atmospheres quiescent enough, for the heavier helium atoms to sink down past the hydrogen atoms to leave a helium-poor atmosphere³. The transition from helium poor to helium rich at an effective surface temperature of about 40,000K indicates either that mixing motions in the star reach up into the atmosphere at temperatures above this, or that the outer layers of the star have been stripped off to leave only the helium core with virtually no hydrogen. Not all the puzzles presented by these stars have been solved; it is not clear how many subdwarfs are close binaries and how mass transfer between the two components of such a binary would affect their evolution. One of the helium-poor subdwarfs is suspiciously near the centre of a supernova remnant (SN1006), suggesting a possible evolutionary connection. Neither is it clear how the precursors of the extremely helium-rich subdwarfs could have lost virtually all their outer hydrogen-rich layers.

Velocity fields in stellar atmospheres are known to exist from their broadening the spectral lines observed in the light from a star, but their nature is poorly understood. The most problematic are the so-called 'microturbulent' velocities which probably have little to do with turbulence, but give velocity variations on a physically small length scale in the atmosphere — hence the epithet 'micro'. The two most likely candidates for microturbulent broadening are either the differential velocity shifts in

convective elements penetrating up from the convection zone and into the stellar atmosphere or some kind of acoustic or magnetoacoustic wave motion. It is possible that both mechanisms contribute to the actual line broadening in stars.

Å. Nordlund (Copenhagen University Observatory) presented beautiful computer simulations of solar convective cells, which calculate the radiation transfer through a hydrodynamic simulation of the flows and reproduce well the broadening and shifts of lines in the solar spectrum. His calculations give a harsh warning to atmospheric analysers by showing that at a given vertical height in the solar atmosphere, a great range (perhaps 5,000K) in temperature can exist at different (but close) horizontal positions. He has also computed preliminary models for the giant star Arcturus, and again obtains line broadening in reasonable agreement with observations, but showing even bigger temperature fluctuations. Further calculations are eagerly awaited.

The alternative model of waves generated in the convection zone and propagating up through the atmosphere, broadening the lines as they go, also has attractions as a possible method of depositing energy in the outer layers of the atmosphere. Such a heat source is required to maintain the observed high temperatures of the chromospheres and coronae of stars. But the energetics of such a process are debatable and difficult to calculate. Simple acoustic waves generated by the Lighthill jet-noise mechanism have the behaviour to account for the observed microturbulent broadening⁴ and have recently been staunchly defended as a possible chromospheric heating source⁵. The true situation, however, may be considerably more complicated. In particular, the Einstein observations⁶ show that nearly all stars have a hot corona which generates X rays, but with a wide variation in emission level even among stars of the same type. It is not yet clear how important the stellar magnetic fields will be in determining the X-ray emission. R.F. Stein⁷ has argued persuasively that the upward energy transport to the chromosphere and corona is predominantly in magnetohydrodynamic waves — particularly Alfvén and slow-mode acoustic — generated where the background magnetic field is strong in the convection zone. This gives a better energy budget than for simple acoustic waves, and might imply a rather 'patchy' line broadening over the surface of a star as a result of the typically inhomogeneous magnetic field structure. It is also possible that the (variable) magnetic field structure of the star modulates the conversion of waves into heat energy and thence to X rays in the upper atmosphere.

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*The workshop was organized by University College, Cardiff. Enquiries about the project may be directed to its secretary, Dr A.E. Lynas-Gray, Department of Physics and Astronomy, University College, London.

In most hot stars there is no convection zone near the atmosphere, so neither convective cells nor wave mechanisms would seem to work, and yet both coronae and microturbulence are observed. Radiative amplification of acoustic waves is a possible explanation⁸ but again the magnetic structure of the star may be important.

The detailed computation of these dynamic and noisy atmospheres is orders of magnitude more time consuming than the usual simple plane-parallel static models. The possible influence of the chromosphere on the lower layers of the atmosphere, effects of sphericity in

extended atmospheres, proper accounting for the thermodynamic disturbances exerted by waves⁹ and relaxation of the local thermodynamic equilibrium condition can all add to the complication. The setting up of a collaborative computing project seems to be a timely move. □

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Terrestrial ages of meteorites

from Derek W.G. Sears

It has taken about 250 years of scanning the popular journals, of writing begging letters, of circulating popularized leaflets and of outright bribery to amass the collection of around 2,000 meteorites that were in the world's museums in 1969. In the last decade or so, while the attention of most of us was focused on the rocks being gathered on the Moon, almost 4,000 meteorites have been discovered in several regions of Antarctica.

The Antarctic discoveries have led to a rekindling of interest in determining how long the meteorites have been on Earth, their so-called terrestrial age. Prosaic reasons for determining terrestrial ages are often offered — for example, it may enable meteorites which are fragments of a single fall to be identified — but when so many meteorites are found over an area of a few thousand km² it seems natural to ask how long they have been accumulating.

Terrestrial age determination is made possible because nuclear reactions are induced in meteorites by cosmic rays. The nuclides produced by such reactions are usually radioactive. If one knows their abundance at the time of fall and their rate of radioactive decay, then it is a trivial matter to calculate the meteorite's terrestrial age from the present abundance of such nuclides. However, the abundance at the time of fall is difficult to determine, and consequently terrestrial age determinations are appreciably less accurate than those of the several other important ages in the life of a meteorite, such as their formation age or the duration of their exposure to cosmic radiation. Usually the abundance of a cosmic ray-produced nuclide is measured in a number of meteorites that have fallen recently, and their

average value is assumed to apply to all the meteorites being dated. In fact, this quantity is affected by many factors, such as the duration of exposure to cosmic rays and the depth of the sample in the meteorite during this exposure, and varies considerably.

Another complication is that the terrestrial age must be within a factor of about 10 of the half life of the activity being used if it is to be determined. If too many half lives have passed since fall, then too little activity will remain to permit detection. If less than a half life has passed, then there will be too little decay to detect. In such cases only a lower limit or an upper limit, respectively, can be determined. Fortunately, the half lives available seem to cover the appropriate range so that with two activities one can frequently bracket the actual value. For example, Fireman *et al.*¹ observed that the Antarctic meteorites were frequently too newly fallen for ²⁶Al dating ($t_{1/2} = 0.72$ Myr) and too old for ¹⁴C dating ($t_{1/2} = 5,720$ yr), and this has proved to be a general rule^{2,3}. From a judicious combination of ³⁶Cl ($t_{1/2} = 0.3$ Myr), ²⁶Al, ¹⁴C and ⁵³Mn ($t_{1/2} = 3.7$ Myr) data, Nishiizumi *et al.*⁴ were recently able to assign terrestrial ages to nine Antarctic meteorites: three had ages of < 0.2 Myr, one of 10⁴ yr and the remainder of 0.13–0.69 Myr, with typical errors of 10–20 per cent.

The thermoluminescence (TL) technique is the latest to be applied to this purpose. The level of TL in a meteorite is dictated by competition between its build up due to exposure to ionizing radiation, and its decay due to thermal draining. In the low-level radiation environment of Earth, TL decays from its high in-space levels to a much lower intensity. Once again, a major uncertainty is the in-space level; freshly fallen meteorites show a wide range in their TL intensity.

In a recent paper⁵ Melcher follows the work of Sears and Mills⁶ and plots TL against terrestrial age for numerous meteorites with known dates of fall. He departs from the earlier authors in normalizing his data differently but the overall picture is unchanged. There is a hint that after about 250 yr some decay in the TL can be detected, but the 250 yr period with available data is clearly far too short for any really convincing trends to appear. How then is one to calibrate the TL decay in terms of terrestrial age? This has proved to be far more difficult than the optimistic efforts of Sears and Mills in 1974 supposed.

Unlike the decay rate of a radioactive isotope, the rate of TL decay is not a well determined, invariable quantity. Only recently has it become apparent that meteorite TL obeys second-order kinetics; that is, logarithm of the TL intensity does not decrease at constant rate, but at a rate proportioned to its intensity. The decay rate is also highly sensitive to the ambient temperature of the meteorite, being higher for higher storage temperatures. Melcher also reports that at 160°C, TL decay can be observed after only a few hours. Using plausible solid-state models for the TL mechanism, he calculates the decay at lower temperatures which can be compared with further annealing experiments. It seemed to this reader that theory and experiment were already parting company when extrapolated from 160°C down to 121°C; even allowing for the very large uncertainties in the data used in the theory. Melcher's data do not bode well for determining the decay curves for Antarctic meteorites from such procedures.

We are left with the rather unsatisfying procedure of calibrating the TL decay using terrestrial ages obtained isotopically. Melcher finds a 'rough correlation' between TL and the ²⁶Al content of nine meteorites and the ³⁶Cl content of another eight. Using 17 meteorites from the Prairie States of the US, where terrestrial ages seem to be appropriate for ¹⁴C dating, Sears and Durrani⁷ found statistically significant correlations between TL and ¹⁴C terrestrial age. Such exercises serve to demonstrate the predicted relationship between TL and terrestrial age. However, until the TL technique can be put into absolute footing, independent of the isotopic techniques, it will not find widespread application, except perhaps, as Melcher suggests, as a 'quick and dirty' method of rapidly screening large numbers of meteorites in order to select interesting ones for study by other techniques. □

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Interactions of biogeochemical cycles

from Bert Bolin

CARBON, nitrogen, phosphorus and sulphur and of course hydrogen and oxygen are needed in precise proportion for the formation of the fundamental molecules on which life depends. Their availability, interactions and circulation in nature have therefore been decisive to development of life on Earth and to maintenance of the present global ecosystem. The development of living organisms in turn substantially modified the primordial distributions and circulation of these basic elements. Since the mid 1970s, the Scientific Committee on Problems of the Environment (SCOPE) has been studying the global cycles of carbon, nitrogen, phosphorus and sulphur, and in particular, the overall balance of the material fluxes due to air and water motion. It is hoped that the long-term impact of man's wastes on the ecosystem can be effectively analysed and possible future changes foreseen. A SCOPE workshop* was held recently outside Stockholm to survey our present knowledge.

In the atmosphere the fundamental role of the hydroxyl radical (OH) formed when oxygen is photodissociated and the exited oxygen atoms react with water, has been firmly established. It oxidizes CH_4 and CO to CO_2 , NO and NO_2 (NO_x) to nitrate and reduced sulphur compounds and SO_2 to sulphate. The C, N and S cycles are thus coupled and their interactions have changed during the evolution of the atmosphere.

Terrestrial ecosystems have been able to establish nearly closed circulation patterns to economize on nutrients, which are often in limited supply. To foresee possible future changes we need to understand how these systems have evolved. In the hydro-sphere running water is an important agent for translocation of all the nutrients on land and for the slow transfer from land to sea. However, an approximate steady state of the N and S cycles has been maintained over the past few millennia by the return flow from the sea to land via air motions.

Human activity is now inducing fluxes of carbon, nitrogen, phosphorous and sulphur comparable in magnitude to natural fluxes. The annual release of CO_2 fossil fuels is about 10 per cent of that used in natural primary production by terrestrial plants. It is not certain how photosynthesis and biomass accumulation on land may change due to this disturbance, and it became apparent at the workshop that the

pathways of the excess CO_2 would be resolved only by examining the interplay of the N and P cycles with the carbon cycle.

The amount of NO_x and NO_3 formed in fossil fuel combustion and fertilizer manufacturing is about one-half of that produced naturally by the biosphere. Nitrogen oxides (NO and NO_2) are important in tropospheric ozone reactions: above a critical NO_x concentration ozone is created as a by-product of the photochemical oxidation of hydrocarbons. Hence tropospheric ozone in the Northern Hemisphere may have increased significantly due to the man's impact on the N cycle. In contrast, the photochemical influence of NO_x on the stratospheric ozone distribution leads to ozone depletion. Enhanced nitrogen loading to terrestrial systems resulting from fossil fuel combustion may increase net primary production (including forest biomass) due to the fertilizing effect of N, and enhanced N and P loading to rivers (see below) is likely to lead to the eutrophication of coastal waters and an increase in organic matter sedimentation.

SO_2 emission to the atmosphere, primarily by fossil fuel combustion, probably exceeds the exchange of sulphur (excluding sulphur in sea spray) by air motions between land and sea. The oxidation of SO_2 to H_2SO_4 and its deposition near main industrial areas is acidifying the lakes of Scandinavia, Canada and the northeastern US, with damaging effects on the biota. Most terrestrial ecosystems are relatively well buffered with respect to pH and direct toxic effects of increased acidity have only been conclusively established in restricted areas. An indirect effect of acidification is the increasing levels of aluminium, which is toxic to roots, in the ground water. Nitric acid, produced from the oxidation of NO_x , has also contributed to the increased acidity of precipitation. The increased input of ammonia, which has taken place simultaneously as a result of growth in agriculture and increased use of nitrogen fertilizers, has only partially balanced the increased acidification due to sulphuric and nitric acids.

Industrial emissions, particularly of SO_2 , have led to a change in visibility of the atmosphere due to the formation of ammonium sulphate. Together with the urban photochemical smog produced from automobiles exhaust gases, in particular NO_x , a dramatic change in the environment is being produced. Chemical interaction of N and S in the atmosphere leads to changes in their atmospheric residence times and will bring about an increase in the size of the affected areas, particularly as fossil fuel burning increases

in the future.

As a consequence of the widespread use of fertilizers, detergents and industrial chemicals containing N and P, the riverine fluxes of these elements seems to have increased about twofold over the past 30 yr resulting in increased biological activity in rivers and coastal zones. The increased supply of organic detritus from plant production in the photic zone will enhance the amount of carbon stored in the sediments. Algae at the base of the food chain are replaced by less desirable species and the areas of anoxia increase. Coastal waters are essential to man both because the human population is concentrated along the shores and because the sea is an important source of food. Our understanding of coastal ecosystems is by no means adequate to judge how man is modifying the waters and ocean floor along the shores.

Present methods of cultivation cause substantial losses of C, N, P and S from terrestrial ecosystems through accelerated decomposition, reduced additions of structural organic material to the soil, increased erosion and the removal of N, P and S in harvested products. However, by using alternative strategies for land management, which balance the net input of organic C, N, P and S, and thereby simulate the interacting nutrient cycles of natural terrestrial ecosystems, loss of soil fertility can be minimized and the organic matter, nutrient content and productivity of degraded sites even increased.

An additional problem is the aging of soils over millions of years associated with the transformation of phosphorus into biologically inaccessible compounds. In the light of marked changes in soils, particularly in the arid and semi-arid regions, long-term strategies for soil management are urgently needed.

Man's effect on the C, N, P and S cycles and their interactions in the global ecosystem are becoming clearer. We have learned that disturbances in one region of the world may affect the world as a whole, but quantitative models of the key biogeochemical cycles are still very crude. In the future, an approach similar to that used in climate modelling needs to be adopted. It is now clear that an understanding of the main features of the general circulation of the atmosphere and the oceans are crucial for modelling the biogeochemical cycles and conversely, climate and climatic change cannot be understood without knowledge of the biogeochemical cycles. □

*The international workshop on the Interaction of biogeochemical cycles took place at Örsundsbro outside Stockholm on 25–30 May 1981 with financial support from UNEP. The outcome of the meeting will appear as SCOPE report (no.24). Reports nos 7, 13 and 16 summarize previous workshops and symposia, and no.17 will soon be published, on the SCOPE General Assembly held in Stockholm in June 1979.

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ARTICLES

Phanerozoic marine diversity and the fossil record

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Strong correlations between various local and global estimates of Phanerozoic marine diversity for taxa below the ordinal level indicate a single pattern of change underlying all data on fossil density. Geological time alone seems insufficient to explain all of the significant covariation among the data sets, and it is proposed that the common pattern in diversity reflects the signal from a real evolutionary phenomenon strong enough to overcome the biases inherent in the fossil record.

THE 'imperfection of the fossil record' has always been the single greatest impediment in the palaeontological study of evolution. Incomplete preservation, inadequate sampling and arbitrary taxonomy have made it difficult to discern and assess accurately large-scale evolutionary patterns in the history of life¹⁻³. For the study of taxonomic diversity, these problems have led to uncertainty about both the patterns and the processes of diversification in terrestrial and especially marine ecosystems. No single data set has even been able to demonstrate conclusively whether species diversity in the oceans has increased substantially since the Palaeozoic era or whether it has remained essentially constant over the past 500 million years^{4,5}. However, when all empirical estimates of Phanerozoic marine diversity are considered together, a remarkably consistent pattern emerges, suggesting that a strong evolutionary signal underlies the preservational and taxonomic noise of the known fossil record.

Phanerozoic diversity

Five major and essentially independent estimates of the diversity of lower taxa in the marine fossil record have been published during the past decade:

(A) Trace fossil diversity—Seilacher⁶ plotted data on observed numbers of ichnospecies in marine facies of various ages at 36 localities from around the world.

(B) Species per Myr—Raup⁷ published data on global numbers of fossil invertebrate species compiled from a '10% sampling' of the 1900–70 volumes of the *Zoological Record*.

(C) Species richness—Bambach⁴, in attempt to avoid biases inherent in estimates of global fossil diversity, compiled data on the numbers of invertebrate species described from 386 marine, level-bottom communities of Cambrian to Quaternary age.

(D) Generic diversity—Raup⁸, for an analysis of taxonomic survivorship, compiled data on the times of origination and extinction of all genera and subgenera listed in the pre-1978 volumes of the *Treatise on Invertebrate Paleontology*⁹.

(E) Familial diversity—Sepkoski¹⁰ published a graph showing the stage-by-stage history of the global diversity of all marine metazoan families, compiled from some 300 literature sources.

These five sets of data on Phanerozoic marine diversity (Fig. 1) have been reduced to the common stratigraphic resolution of this geological system and plotted on a normalized vertical scale. The data for trace fossil diversity may be more heterogeneous than the other four estimates; Seilacher⁶ has argued that trace fossil communities in shallow and deep water are decoupled and hence have had separate histories of diversification. We have combined his data from both neritic (shelf) and flysch (continental slope and rise) facies, however, so that trace fossils as a whole can be compared with data sets on skeletal body fossils.

Correlation analysis

The extent to which all five estimates of fossil diversity are influenced by a common underlying factor can be assessed using simple correlation analysis. Table 1 lists linear correlation coefficients for all pairs of the diversity estimates. All the correlations are quite high, with 8 out of 10 ranging from 0.91 to 0.99. The only two coefficients lower than 0.90 involve the data on trace fossils. These data, as well as being heterogeneous, measure local behavioural diversity, thereby only indirectly reflecting local species diversity⁶, and encompass fewer observations than the other data sets. In contrast, Raup's two data sets, which are based on the greatest numbers of observations, exhibit the highest average correlations.

If standard one-sided statistical tests are applied to the correlation coefficients in Table 1, all appear to be significantly greater than zero at the 0.05 confidence level (and all but the lowest at the 0.001 level). However, these test results cannot be considered valid. Because taxonomic diversity in any interval of geological time is partially dependent on the diversity in the preceding interval¹¹, the usual statistical assumptions regarding independence of samples are violated^{12,13}. To avoid this difficulty, correlations independent of time must be examined.

That all five estimates of Phanerozoic diversity are strongly correlated with time (Table 2) reflects in part the necessary increase in diversity from the earliest Phanerozoic to the present. However, in no case does this correlation with time account for more than ~60% of the variance in estimated diversity. When this correlation is removed, the residual estimates of diversity remain highly intercorrelated, as indicated by both the linear and the rank correlation coefficients in Table 2. One-sided statistical tests indicate that all but the lowest one or two coefficients in either set are significantly greater than zero at the 0.05 level or better.

This last result is important when the variety of uncertainties and biases inherent in each set of data is considered. For

Table 1 Matrix of linear product-moment correlation coefficients between estimates of standing marine diversity in each Phanerozoic geological system

A Trace fossils	(9)	—				
B Species per Myr	(10)	0.907	—			
C Species richness	(8)	0.725	0.929	—		
D Generic diversity	(10)	0.927	0.986	0.912	—	
E Familial diversity	(10)	0.871	0.928	0.927	0.939	—
		A	B	C	D	E

Numbers of systems with data are listed in parentheses. The first relative eigenvalue ($\lambda_1/5$) of this matrix is 0.925, indicating a strong overall correlation among the five diversity estimates.

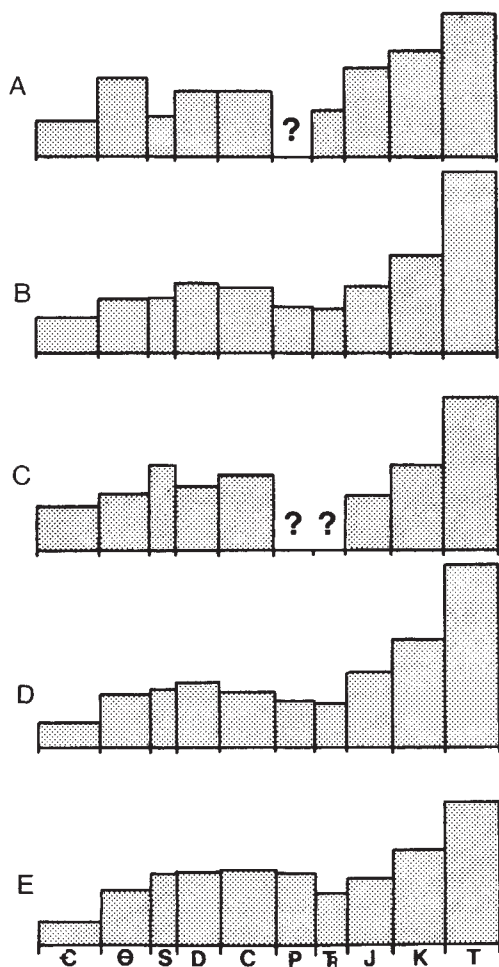


Fig. 1 Histograms displaying the relative magnitudes of marine animal diversity per geological system as indicated in five data sets published during the last decade. The geological systems are denoted with standard symbols beneath the bottom histogram. The heights of the histogram bars have been scaled so that each data set shows the same average diversity. Question marks signify systems for which adequate data are lacking. The data were compiled by the following procedures: (A) Seilacher's⁶ data on trace fossils were averaged by calculating median numbers of ichnospecies per locality in each system within both neritic and flysch facies (with a more recently published²⁹ data point for the Jurassic included); no data were available for the Permian system. (B) Raup's⁷ raw data on numbers of species per geological system were modified by first eliminating the non-marine Insecta and then normalizing the remaining data by dividing by the duration of each system. (C) Bambach's⁴ data on species richness were taken directly from his Fig. 5, which lists median numbers of species in communities in 'open marine environments'; these communities, which constitute the bulk of Bambach's total data set as well as of the entire marine fossil record, comprise the only data that Bambach listed at the level of system (with adequate data for the Permian and Triassic systems lacking). (D) The data on generic diversity were reduced from Raup's⁸ table of numbers of generic originations and extinctions per geological system using a modified Webb-Harper transformation^{30,31}; average standing diversity in each system was calculated as the number of genera passing entirely through the system, plus half the new genera surviving beyond the system, plus half the old genera becoming extinct in the system, plus the number of genera confined to the system multiplied by a factor scaling for the system's duration. This scaling factor was computed as 14 Myr divided by the duration of the system, where 14 Myr (or half the average duration of a genus⁸) was arbitrarily chosen assuming that genera confined to single systems should have durations somewhat shorter than average. (Experimentation with numerators ranging from 10 to 20 Myr altered results by <10%.) (E) The data on familial diversity were generated by weighting the standing diversity of families in each stratigraphic stage in Sepkoski's¹⁰ original data by the stage's duration and then averaging over each system to produce a simple weighted mean.

example, not only are the data on trace fossil diversities heterogeneous with respect to environment, but they may also be heterogeneous with respect to taxonomic level. Some ichnospecies may even represent mixtures of phyla, such as species of *Skolithos* which may have been produced by both phoronids and annelids. The data on species richness were derived from a more uniform set of open shelf environments but may still reflect considerable environmental variation; species richness in benthic communities today varies by more than an order of magnitude within analogous habitats in different climatic zones. Such variation may be confounded further in the fossil record by enrichment of local diversity by lateral mixing¹⁴ and time averaging¹⁵. The compilations of global diversities of fossil species, genera, and families avoid some of these local problems but are subject to a wide range of additional biases which result from variations in the quantity and quality of the geological record, reliability and accessibility of taxonomic literature, number of active workers, and so on^{3,16}. The effects of these biasing factors may vary considerably with taxonomic level. Also important is the differing taxonomic coverage of the various data sets; the familial data in Fig. 1, for example, exclude protozoans, which are incorporated into the species and generic data, but include marine vertebrates, which do not appear in the other two data sets.

Patterns

The strong similarities among the data sets lead us to conclude that all five estimates of Phanerozoic taxonomic diversity are measuring a single underlying pattern that is reflected at both local and global scales and at various taxonomic levels in the marine fossil record. Judging from the elements shared by all data sets, this common pattern is: (1) low diversity during the Cambrian period; (2) higher but not persistently increasing diversity through the post-Cambrian portion of the Palaeozoic era; (3) low diversity in the early Mesozoic era (especially the Triassic period); (4) increasing diversity through the Mesozoic leading to a Cenozoic (Tertiary) maximum.

Bambach⁴ recognized essentially the same pattern in his observation of 'multiple equilibria' in his data on species richness. Differences principally involve transitional diversities: he placed the Ordovician, which is probably transitional between the Cambrian and later Palaeozoic diversities^{10,11}, with the Cambrian, and he included the Jurassic and Cretaceous, which are transitional between the Triassic low and the Cenozoic high, with the Palaeozoic. Similar patterns were also indirectly recognized by Raup¹⁷ and especially Sepkoski^{18,19} in analyses of sampling biases in Raup's⁷ species data. Multiple regressions of species diversity on measures of sampling efficiency (specifically global sedimentary rock volume or outcrop area) and of expanse of epicontinental seas generated consistently negative residuals for Cambrian and Triassic diversities and positive residuals for Tertiary diversity; these residuals correspond to the lows and high reflected in all data sets in Fig. 1.

Table 2 Correlations removing the influence of time from the estimates of Phanerozoic marine diversity

	Time					
A Trace fossils	-0.784	—	0.667 (0.214)	0.611 (0.444)		
B Species per Myr	-0.708	0.783	—	0.571 0.867	0.644	
C Species richness	-0.683	(0.352)	0.885	—	0.714 0.714	
D Generic diversity	-0.771	0.805	0.978	0.888	—	0.689
E Familial diversity	-0.767	0.676	0.851	0.900	0.850	—
		A	B	C	D	E

The column labelled 'Time' lists linear product-moment correlation coefficients between the diversity estimates and the ages of the geological systems, measured as the time from the Recent (0 Myr) to each system's midpoint. The matrix to the right lists partial linear correlations (below) and Kendall's rank correlations (above), obtained by correlating residuals from linear regressions of diversity estimates on time. Coefficients enclosed in parentheses are not significantly greater than zero at the 0.05 confidence level.

Older estimates of Phanerozoic taxonomic diversity also exhibit patterns commensurate with the data sets in Fig. 1. For example, Sepkoski's data set on familial diversity is pre-dated by comparable series-level data published by Newell²⁰ and Valentine²¹. These latter data sets, which were based on an older taxonomic and stratigraphical literature, still correlate highly with Sepkoski's familial data at the series level; Newell's data (with terrestrial vertebrates removed using information from Romer²²) correlate with a magnitude of 0.934 (partial $r = 0.880$ with time removed), and Valentine's data correlate with a magnitude of 0.953 (partial $r = 0.942$). Even the oldest compilations of diversity in the fossil record bear strong resemblance to the modern estimates. Data taken from the Phanerozoic diversity curve published by Phillips²³ in 1860 possess an average correlation of 0.869, and an average partial correlation with time removed of 0.693, with the five data sets in Fig. 1. These relationships suggest that while increased systematic and stratigraphic knowledge may have enhanced the accuracy and resolution of diversity data (see, for example, ref. 24), time has not substantially altered the fundamental patterns seen in the data.

The only estimates of fossil marine diversity that do not show

patterns like those in Fig. 1 involve taxonomic categories above the familial level. Ordinal data sets published by Simpson²⁵, Valentine²¹ and Sepkoski²⁶ show low numbers of orders in the Cambrian and no strong trends through the later Palaeozoic, but none shows a marked increase in diversity in the post-Palaeozoic; in fact, Valentine's data show a major decline in numbers of orders following the Palaeozoic. The reasons for these differences are not entirely clear but may involve the tendency for morphologically distinct groups of animals to appear rapidly during the early phases of major evolutionary radiations^{2,27}; such groups tend to become extinct rather quickly if they fail to diversify internally^{10,28}. Thus, as Valentine²¹ has argued, the magnitude of post-Cambrian diversity portrayed in the ordinal data sets may indicate more the variety of distinct morphological types produced during the early Palaeozoic radiations rather than the actual patterns of low-level taxonomic diversity that seem to be reflected in Fig. 1.

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Palaeontological documentation of speciation in Cenozoic molluscs from Turkana Basin

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A recently discovered series of mollusc faunas from the late Cenozoic of the eastern Turkana Basin constitutes one of the best documented metazoan fossil sequences. Evolutionary patterns in all lineages conform to the 'punctuated equilibrium' model; no 'gradualistic' morphological trends occur. These faunas provide the first fine-scaled palaeontological resolution of events during speciation: fundamental phenotypic transformation of both sexual and asexual taxa occurs rapidly, in comparatively large populations, and is accompanied by a significant elevation of phenotypic variance. This increase in variance reflects extreme developmental instability in the transitional populations.

THE 400 m sequence of late Cenozoic deposits east of Lake Turkana in northern Kenya, discovered¹ by Leakey in 1968, comprises the Plio-Pleistocene Kubi Algi, Koobi Fora and Guomde Formations, and the Holocene Galana Boi Beds². In addition to important palaeoanthropological¹, archaeological³ and vertebrate palaeontological⁴ material, these deposits have recently yielded a uniquely well documented sequence of lacustrine mollusc faunas. The faunas have important implications for present evolutionary controversies, as they provide the first detailed palaeontological documentation of events during allopatric speciation.

Mollusc faunas are scattered throughout 1,000 km of exposures east of Lake Turkana and occur in laterally extensive lensoid accumulations 0.001–1 m thick. These accumulations have a matrix ranging from coarse silt to coarse sand grade, and are separated by finer-grained intervals devoid of molluscs. The

190 faunas reported here consist of various prosobranch, pulmonate and bivalve lineages, and represent both life and death assemblages in various shallow lacustrine and pro-deltaic settings.

Various features make this sequence particularly useful for investigating evolutionary patterns: the molluscs are well preserved and abundant, the units in which they occur are generally unconsolidated, and because most of the species lineages in the section are still extant, reasonable inferences can be drawn concerning the 'soft' biology of their fossil representatives from the Turkana Basin sequence. In biological and taxonomic terms, these mollusc lineages comprise an extremely heterogeneous assemblage (see Table 1); this heterogeneity allows useful comparisons to be made of evolutionary patterns in taxa varying widely in autecology, reproductive strategy and size. In particular, evolutionary patterns in both sexual and

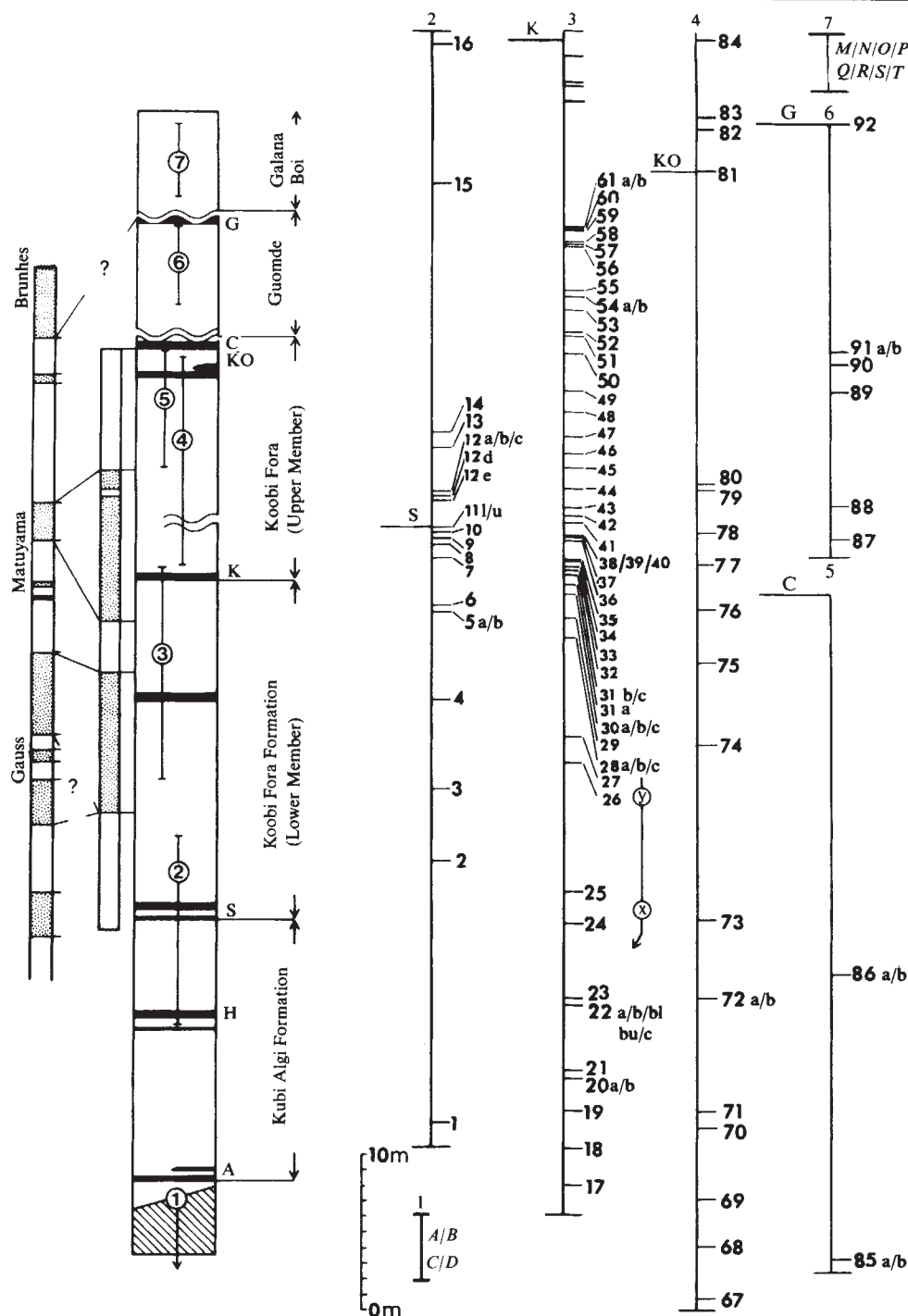


Fig. 1 Generalized chronostratigraphy of late Cenozoic deposits of the eastern Turkana Basin and stratigraphical distribution of mollusc faunas. Geophysical age data and generalized stratigraphy are from various sources^{2,12,38-42}. On the extreme left is shown the world geomagnetic polarity time scale; to the right of this is the polarity sequence observed in the eastern Turkana Basin. Generalized stratigraphical column shows principal tuff horizons and (to the right) ³⁹Ar/⁴⁰Ar or K/Ar age determinations of the tuffs. A, Alia tuff; H, Hasuma tuff; S, Suregei tuff; G, top Guomde tuff. Numbered subsections on the generalized stratigraphical column indicate the general position of the expanded sections numbered 1-7 in the right half of the diagram. These latter sections show the stratigraphical order of mollusc faunas; the metre scale refers to sections 2-6. Faunas A-D are from deposits thought to pre-date the Kubi Algi, or to correspond to a lower level of that unit (fauna A, Casa Waterhole section⁴³; fauna D, deposits at Sibiloit⁴⁰; faunas C, D, Warata Formation⁴⁴). The relative stratigraphical order of these faunas is unknown, as is the order of faunas M-T from the relatively thin Galana Boi Beds. The stratigraphical order of faunas 1-92 is determined by the distance of their upper surfaces from the bases of the named tuff units, as measured in the various local sections. Faunas X and Y come from low down in the Lower Member, but their precise position is unclear. Most of the faunal units are <0.25 m thick. a, b, c and so on denote faunas that are demonstrably sampled from the same mollusc-bearing unit, but from different localities; earlier letters in the alphabet denote more southerly locations. l and u denote samples from the basal and upper thirds, respectively, of a given faunal unit.

asexual taxa can be compared. The 19 species lineages in the section represent 18 genera and 12 families, thus ancestor-descendant relationships between species lineages and their derivative taxa are unambiguous. Because the molluscan shell accretes terminally and is normally unmodifiable after deposition, each shell is a comprehensive record of individual development, and it is therefore unnecessary to construct mass curves to study changes in patterns of ontogeny during evolution. A useful general scheme for the quantification of shell form is available⁵. Finally, previous extensive geological investigations in the Turkana Basin mean that the mollusc sequence can be studied within a well documented chronostratigraphical and palaeoenvironmental context. A comparative evolutionary study can be made within a quasi-experimental context.

Collection and treatment of material

Of the 19 species lineages, 13 were common enough for a biometric analysis. 2-10 kg of shell-bearing sediment were

extracted from each fauna, from which I selected random subsets of individuals representing the various species lineages. 20-30 individuals of each lineage in the sample were usually measured but shell breakage sometimes reduced this number.

Images of selected shells were projected onto a System-2 D-MAC digitizing table, and 5-24 (average 18) measurements, depending on the lineage studied, were made for each shell. These measurements included the cartesian coordinates of specific points on the shell, and the areas, perimeters and geometric centroids of the generating curve and muscle scars. 5-20 (average 15) parameters were derived for each individual from the original measurements, and formed the basis of the biometric analysis and included approximations to Raup's parameters⁵ *W* (whorl expansion rate), *T* (translation rate) and *D* (distance of generating curve from coiling axis), all taken at 2-rad intervals down the coiling axis of each shell. Aspects of Raup's parameter *S* (shape of generating curve) and details of shell sculpture were also recorded; for the bivalves, the derived

Table 1 Primary biological properties of mollusc species lineages from the Turkana Basin sequence

Infaunal	Epifaunal	Deposit feeder	Suspension feeder	Gonochor-istic (internal fertiliza-tion)	Gonochor-istic (external fertiliza-tion)	Facultative herma-phrodite	Permanently thelyto-kous	Modal adult length (mm)		
	x	x		x				30	<i>Bellamya unicolor</i> *	
	x	x		x				65	<i>Pila ovata</i>	
	x	x				x		4	<i>Valvata</i> sp.*	
	x	x		x				2.5	<i>Gabiella senaariensis</i> *	Prosobranchs
	x	x		x				17	<i>Cleopatra ferruginea</i> *	
x		x					x	15	<i>Melanoides tuberculata</i> *	
	x	x				x		8	<i>Bulinus truncatus</i> *	
	x	x		x				55	<i>Gyraulus costulatus</i>	Pulmonates
	x	x		x				3	<i>Burnupia</i> sp.	
x			x		x			35	<i>Caelatura (Caelatura) bakeri</i> *	
x			x		x			30	<i>Caelatura (nitia) monceti</i> *	
x			x		x			16	<i>Pseudobovaria</i> sp.*	
x			x		x			160	<i>Aspatharia arcuata</i>	
x			x		x			110	<i>Mutela nilotica</i> *	Bivalves
x			x		x			100	<i>Pleiodon</i> sp.*	
	x		x		x			140	<i>Etheria elliptica</i>	
x			x		x			14	<i>Corbicula consobrina</i> *	
x			x		x			2	<i>Pisidium (afropisidium) pirothi</i>	
	x		x		x			7	<i>Eupera ferruginea</i> *	

Data are from various sources.

* Taxa used in the biometric analyses.

parameters also included descriptions of shape, relative size and disposition of the muscle scars and hinge features. Some 3,300 individuals representing 13 lineages were measured. Canonical variate and principal component analyses were performed on the derived parameters for each species lineage.

The Turkana Basin sequence is punctuated by a prominent series of tuffaceous horizons. The bases of these tuffs are isochronous, laterally extensive and mappable⁶, and they form the basis of the regional chronostratigraphic framework². Mollusc faunas were therefore stratigraphically ordered by surveying them into tuff bases. This ordering of the faunas assumes that sedimentation rates in the contiguous areas from which they were collected were similar—apparently the case—and that the various tuffs have been correctly correlated throughout the areas of exposure. Some correlations between tuffs have been questioned on the basis of (vertebrate) biostratigraphical evidence^{7,8}, but these problematic correlations are not generally in areas yielding the molluscan faunas.

Patterns of evolutionary change

Despite the variation in autecology, reproductive strategy and size of the lineages, their patterns of evolutionary change are fundamentally identical. Figures 2 and 3 summarize aspects of this pattern for the prosobranch *Bellamya unicolor* (Olivier). Canonical variate analysis indicates that populations from the Kubi Algi and pre-Kubi Algi, from most levels in the Koobi Fora Formation and from the Galana Boi Beds group together (Fig. 2). All individuals from these populations are readily referred to the extant species *B. unicolor*. Despite the long-term stasis shown by the *B. unicolor* lineage during the later Cenozoic, three stratigraphically circumscribed but important morphological excursions occur during this period: at the Suregei tuff level, in the Lower Member of the Koobi Fora Formation and in the Guomde. At the Suregei tuff (Fig. 1), populations of *Bellamya* from faunas 12b and 12c consist exclusively of a novel and highly distinct form. Populations morphometrically intermediate between this unusual form and typical *B. unicolor* occur in faunas 8 and 11, just below the base of the Suregei tuff. Another morphological excursion occurs in faunas 27 and 29 in the Lower Member of the Koobi Fora Formation; here a second novel form of *Bellamya* occurs together with typical *B. unicolor*. A third excursion is documented by fauna 88 in the Guomde, where populations consist exclusively of a third novel form of *Bellamya*. Although Fig. 2 suggests that the Guomde population resembles that of the aberrant Suregei tuff level, it is in fact displaced away from them along the third canonical variate; in terms of generalized distance (Mahalanobis' D^2), the Guomde population is equidistant from the latest Suregei level

Bellamya (6.25 units from population 12c) and the nearest typical *B. unicolor* population (6.35 units from population 30). Intermediates between typical *B. unicolor* and the divergent *Bellamya* morphs in faunas 27 and 29, and in fauna 88, are unknown.

As suggested by Fig. 2 and confirmed by comparisons with museum collections of recent *B. unicolor* material, all three novel morphs of *Bellamya* lie outside the narrow phenotypic range of *B. unicolor*. The profound differences in shell geometry of these divergent forms are at least as great as those characteristic of different extant *Bellamya* species. However, the presence of the intermediate forms at the Suregei tuff level and the absence of other potentially ancestral forms of *Bellamya* in the late Cenozoic of north-east Africa indicate that all three divergent morphs of *Bellamya* in the section are derived from *B. unicolor*, although the details of this derivation are only documented at the Suregei tuff level.

Figure 3 shows aspects of a principal component analysis of the morphological excursion in the *B. unicolor* lineage at the Suregei tuff level. As suggested by the canonical variate analysis, populations from faunas 8, 11 and 12 show rapid movement away from typical *B. unicolor* morphology. There are also significant changes in phenotypic variance during this period of morphological transformation. The intermediate population 8, the centroid of which is 6.66 generalized distance units away from the latest divergent *Bellamya* population (12c), shows substantial overlap with both typical *B. unicolor* and the latest derivative *Bellamya* morphs (Fig. 3). However, population 8 displays a striking elevation of phenotypic variance (the summed variance of all measured parameters). Total phenotypic variance in population 8 is 45.5, which is significantly greater at the 2.5% level (one-sided *F*-test)¹⁰ than that of populations of typical *B. unicolor* from elsewhere in the section (average variance 9.1, range 5.4–12.3). As discussed below, increase in phenotypic variance reflects major developmental instability in this transitional population. Population 11, 4.39 generalized distance units away from the latest derivative *Bellamya* of fauna 12c, shows greater overlap with this population than fauna 8, but less overlap with typical *B. unicolor*. However, population 11 has a phenotypic variance (12.7) comparable with that of typical *B. unicolor*, despite its intermediate morphology. The latest divergent population from the Suregei tuff level, from fauna 12c, is ~6.9 generalized distance units from the nearest population of typical *B. unicolor* (population 30) and forms a completely non-overlapping cluster with typical *B. unicolor*; however, the variance (18.5) of fauna 12c is comparable with that of typical *B. unicolor*. The general picture of events in the *Bellamya* lineage at the Suregei tuff level is one of rapid morphological

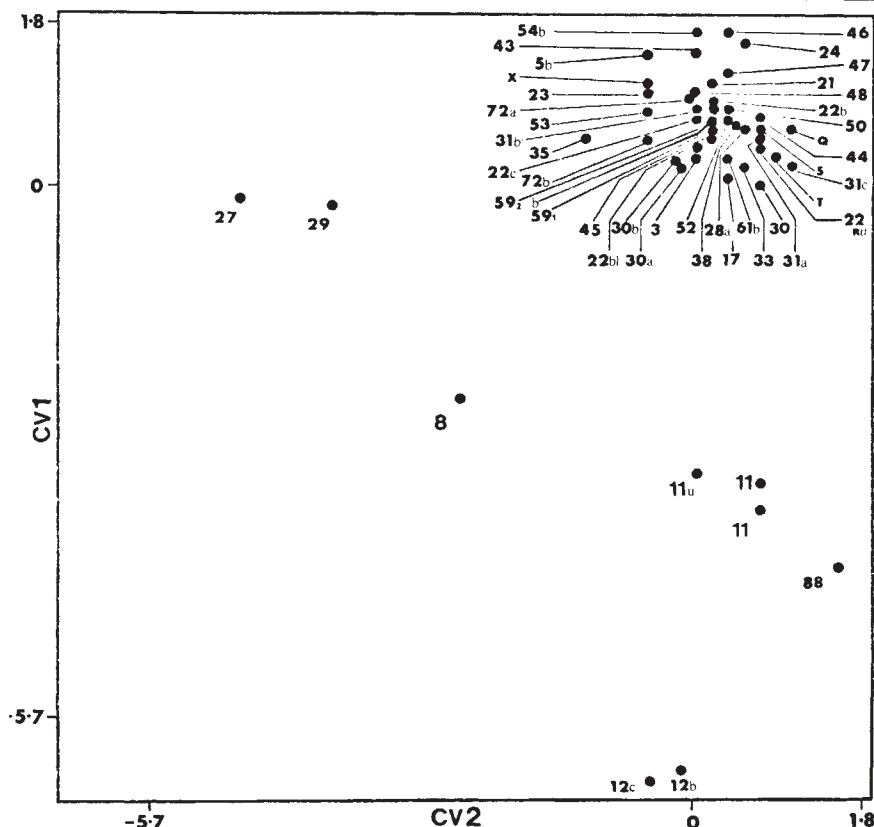


Fig. 2 Summary of canonical variate analysis of 49 populations of the *B. unicolor* species lineage from the late Cenozoic of the Turkana Basin. Analysis based on 16 parameters derived from 16 original measurements, for each of 761 individual specimens. Population centroids are plotted on to the first two canonical variates (CV1 and CV2), which together explain ~60% of the total variance. Population numbers correspond to fauna numbers in Fig. 1. (See discussion in text.)

transformation, initially accompanied by a major increase in phenotypic variance. However, faunas overlying this period of transformation (fauna 13 and above) yield populations of typical *B. unicolor*.

The general pattern of phenotypic change exhibited by the *B. unicolor* lineage is summarized in Fig. 4, which also indicates similar patterns for all other well documented mollusc lineages. Major phenotypic transformations occur simultaneously in all lineages at the Suregei tuff level. For *B. unicolor*, *Melanoides tuberculata* and *Caelatura bakeri* this change involves a significant initial increase in phenotypic variance (in fauna 8 in *Bellamya* and *Caelatura*, and in faunas 8, 9 and 11 in *Melanoides*). Subsequent transformation is accompanied by reduced levels of phenotypic variance, comparable with those of typical ancestral populations of the species lineage. In *Gabbiella senaariensis* and *Cleopatra ferruginea* the peak of phenotypic variance does not occur until the latest divergent faunas (faunas 12a–d) and its subsequent decline is therefore not documented. A major phenotypic transformation is documented for *Pseudobovaria* sp. A., *Caelatura monceti*, *Corbicula consobrina*, *Mutela nilotica* and *Eupera ferruginea*, but populations intermediate between typical representatives of these species lineages, and their distinctive derivatives at the Suregei tuff level, are too poorly documented for analysis of change in phenotypic variance. By fauna 12, all lineages present have transformed such that, in terms of individual morphology, they constitute completely non-overlapping clusters with the typical ancestral forms: the fauna as a whole is unique and endemic to the Turkana Basin. A similar series of events occurs in the Guomde Formation: all representatives of the various species lineages are endemic and distinct, but the angular unconformity between the uppermost Koobi Fora and the Guomde precludes documentation of intermediate forms.

As indicated in Fig. 4, immediately above the Suregei tuff level there is an abrupt reversion to ancestral morphology in all species lineages. No intermediates are known. Subsequently, in the Lower Member of the Koobi Fora, there is a minor adaptive radiation in several lineages. Novel endemic forms suddenly enter the record and coexist with their parent forms. However, in *Melanoides*, various novel morphs arise sympatrically, via

intermediate forms. This phenomenon, described in detail elsewhere¹¹, apparently represents a gradual clonal radiation in this asexual form. All novel Lower Member lineages are terminated by climatically induced regression^{11,12} and the consequent increase in alkalinity^{13,14} in the uppermost Lower Member. Intralacustrine endemic radiations such as that documented in the Lower Member of the Koobi Fora are known from many modern rift lakes^{15,16}.

Implications

A persistent problem in evolutionary biology has been the absence of intermediate forms in the fossil record. Long-term gradual transformations of single lineages are rare¹⁷ and generally involve simple size increase or trivial phenotypic effects^{17–20}. Typically, the record consists of successive ancestor-descendant lineages, morphologically invariant through time and unconnected by intermediates. Eldredge and Gould¹⁷ have suggested that this 'punctuated equilibrium' geometry of phylogeny is a logical outcome of several postulated allopatric speciation mechanisms, particularly Mayr's²¹ 'founder effect' speciation model. This model considers that homeostatic mechanisms and gene flow prohibit significant evolutionary change in large panmictic species populations, but in small, stressed, geographically isolated populations, homeostatic mechanisms break down during 'genetic revolution' and rapid evolution may ensue²¹. The abrupt entry of new lineages into the fossil record therefore represents immigration from isolated sites of origin; the small size and ephemerality of the populations in which cladogenesis occurs normally preclude palaeontological documentation of speciation and associated intermediate forms¹⁷. The phylogenetic geometry of molluscan lineages from the Turkana Basin sequence clearly conforms to the punctuated equilibrium model: long-term stasis in all lineages is punctuated by rapid episodes of major phenotypic change. No 'gradualistic' morphological trends¹⁷ occur in any lineage. The long-term temporal stasis in phenotype documented in both sexual and asexual lineages is paralleled by the geographical stability in phenotype exhibited by their widely distributed modern representatives; shell form in the lineages studied is normally highly 'heritable' (the extreme ecophenotypic variability of

freshwater Bassomatophora is not typical of the gastropods studied here, most of which are prosobranch species characterized by narrow phenotypic ranges in modern faunas).

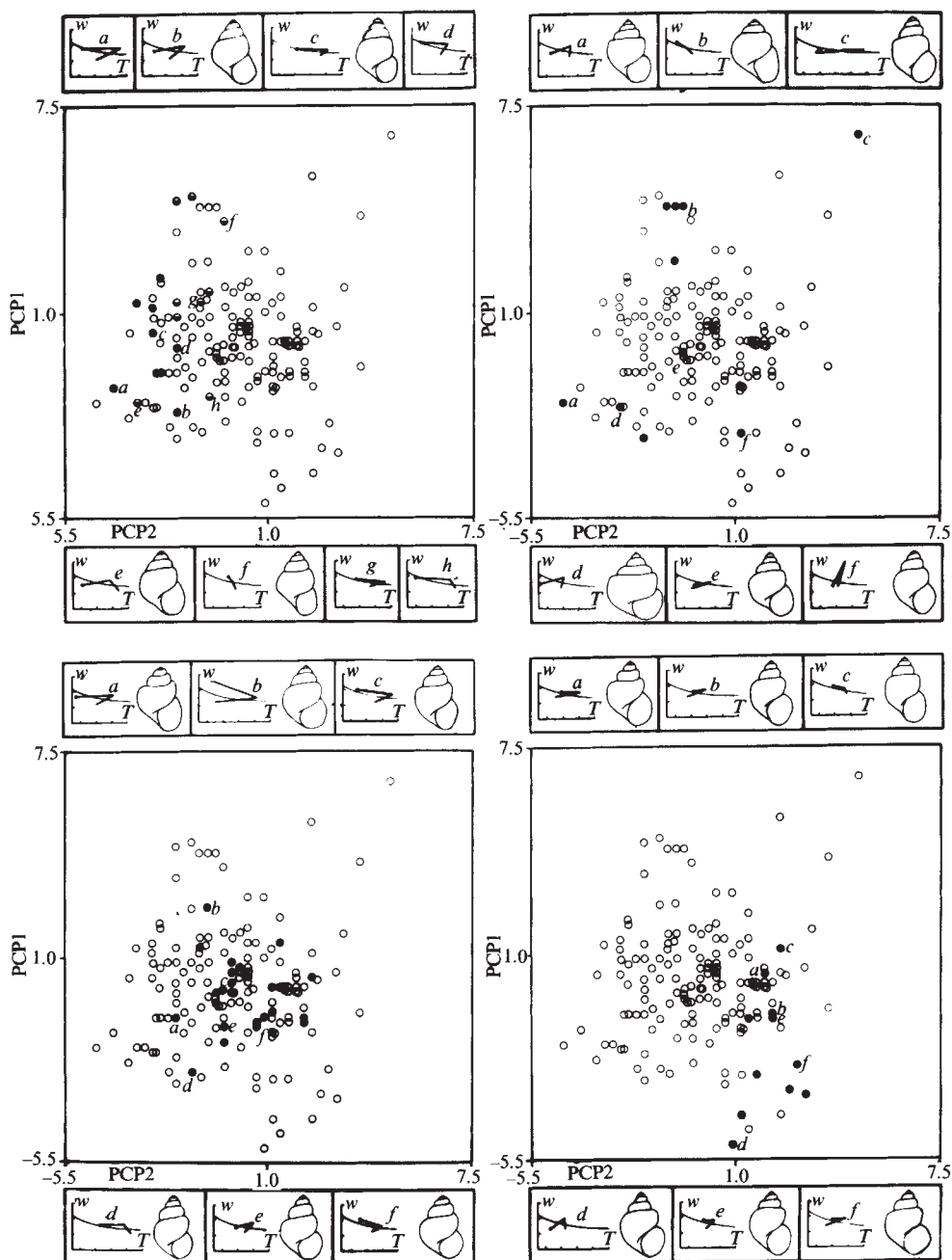
Events during the radiation of several lineages in the Lower Member of the Koobi Fora are typical of the fossil record in that intermediates between derivative and ancestral taxa are not documented. However, events at the Suregei tuff and Guomde levels are significant in that they provide, for the first time, details of allopatric speciation during the 'punctuation' of cladogenesis; they allow an unprecedented resolution of the fine structure of events during speciation. Previous documentation of phenotypic divergence within lineages has involved sympatric stocks and has been interpreted as long-term character displacement after unrecorded allopatric speciation events (for example, see refs 22, 23). Although Ovcharenko²⁴ has claimed to document allopatric derivation of the brachiopod *Kutchithyris euryptycha* from *K. acutiplicata* in a Bathonian section in the Pamirs, no biometric data are available for this supposed transition and the significance of these faunas is unclear.

The events at the Suregei tuff and Guomde levels clearly document speciation within peripheral isolates. Major phenotypic transformations, of at least as great a magnitude as those

now characterizing different extant biological species of the genera concerned, occur almost simultaneously in all lineages at these levels, but typical, unaltered representatives of the various ancestral lineages are known from other, contemporaneous East African sites throughout the late Cenozoic¹⁰. The phenotypic shifts at the Suregei tuff level are continuous, unidirectional and must (from general section thickness estimates) have taken 5×10^3 – 5×10^4 years to accomplish¹⁰. I believe that these considerations, together with the long-term temporal stasis in morphology exhibited by these lineages and their current morphological stability in a diverse range of modern environments, preclude the possibility that such dramatic phenotypic shifts are in any sense 'non-genetic' or ecophenotypic.

Although several aspects of Mayr's 'founder effect' model²¹ of speciation have been challenged recently^{25–27}, the pattern of phenotypic change at the Suregei tuff level—rapid directional change in morphology initially accompanied by extreme developmental instability—agrees with important aspects of Mayr's model. As indicated above, Mayr²¹ emphasized both geographical isolation and environmental stress as important 'triggers' for speciation. Speciation events at both the Suregei and Guomde levels coincide with major lacustrine regressions,

Fig. 3. Summary of principal components analysis of individuals of the *B. unicolor* species lineage from populations at and below the Suregei tuff level, represented by open circles, plotted on to the first two principal components (PCP1 and PCP2), which together explain ~45% of the total variance. ● And ○ represent individuals from five successive faunas spanning the marked phenotypic transformation documented at the Suregei tuff level. *a*, Individuals from fauna B (●) and from fauna 3 (○); *b*, individuals from fauna 8b (●); *c*, individuals from fauna 11 (●); *d*, individuals from fauna 12c (●). Note the rapid shift in phenotype from the ancestral *B. unicolor* morphology (in the left-hand half of the plot) to the divergent form from fauna 12c (to the right). Note also the initial increase in phenotypic variance during this transformation, reflected in the wide scatter of individual morphologies from population 8b. The bivariate diagrams above and below the plots show successive ontogenetic values of *W* (whorl expansion rate) and *T* (translation rate)⁸ for selected representative individuals. The curve shown is the function $T = 2\sqrt{W}/(W-1)$. The solid point at the end of each *W/T* track indicates the earliest measured point in ontogeny. These tracks record *W* and *T* values at three successive, equivalent, 2π radian intervals down the shell (over the last four whorls). Individuals to which the *W/T* plots and sketches refer are identified on the PCP plots by the letters *a*, *b*, and so on. Note the derangement of the *W/T* tracks in the phenotypically variable intermediate population 8b. (See discussion in text.)



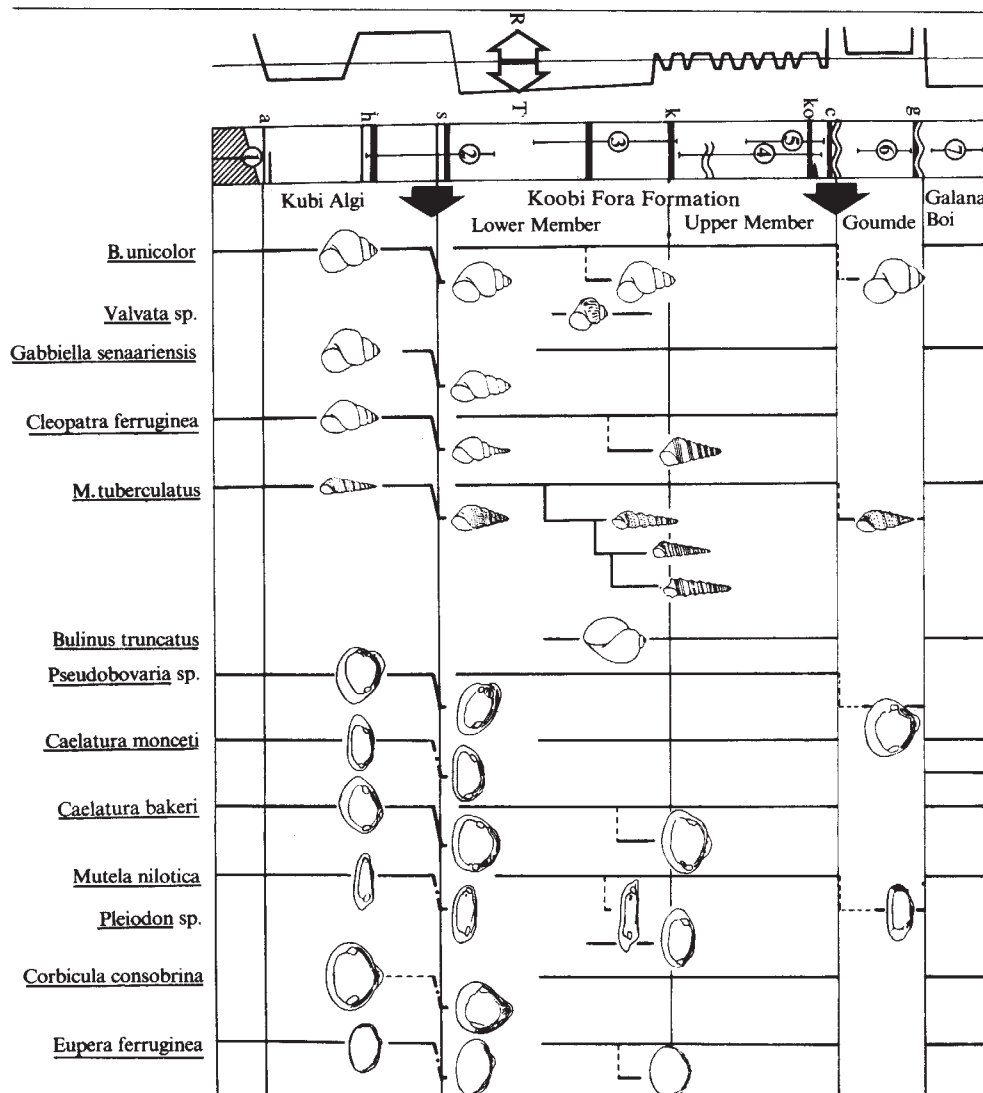


Fig. 4 Generalized summary of patterns of evolutionary change in the Turkana Basin mollusc sequence. On the left is a generalized stratigraphical section as in Fig. 1. The heavy line on the extreme left of the diagram is a generalized 'transgression (T)-regression (R)' line based on various sources^{2,11-14}. The lighter vertical line corresponds to a lake level which is largely the same as the present level. Heavy vertical lines in the main part of the diagram indicate the time ranges of the various species lineages. Broken heavy lines at the Suregei tuff level in some lineages indicate poorly documented speciation events. Only the commonest morphologies developed during the extensive sympatric radiation of the asexual form *Melanoides tuberculata* in the Lower Member are indicated. Some species lineages are not known from the lower- and mid-Kubi Algi Formation, but are known from either pre-Kubi Algi sections in the eastern Turkana Basin, or from the Mursi Formation in the Omo sequence to the north of the basin (D. Vandamme, personal communication). Biostratigraphical evidence (P.G.W., unpublished results) indicates that the latter sequence overlaps with the lower- and mid-Kubi Algi. Although *Cleopatra ferruginea* is unknown from the Galana Boi, this species is extant in other areas of East Africa. Note that sketches of representative shells are not all drawn to the same scale. (Species lineages whose trivial names are not given (sp.) are now being described by Dr D. Vandamme.) The principal evolutionary events documented in the section are (1) the simultaneous speciation events in all lineages at the Suregei tuff and Guomde levels of the basin (indicated by heavy arrows) and (2) the adaptive radiation of several stocks in the middle part of the sequence (Lower Member of the Koobi Fora Formation).

when faunas in the basin are likely to have been both isolated and under stress. Stunting of faunas immediately before the Suregei level and the small number of lineages in the Guomde may reflect stress at these levels. Lacustrine transgression after these regressive phases coincides with reinvasion of ancestral stocks into the basin and elimination of the derivative taxa (Fig. 4): Mayr considers this pattern of events to be the common fate of new species in geographical isolates²¹. In addition, increase in phenotypic variance during allopatric speciation, as documented at the Suregei tuff level, has been predicted as a consequence of Mayr's founder effect model by Levins²⁸, who suggests that developmental instability, due to breakdown in canalization of individual ontogeny, is a likely concomitant of disruption of homeostatic mechanisms during the 'genetic revolution' accompanying speciation (see also refs 26, 27, 29). The molluscan shell is a terminally accreting structure, and the increase in phenotypic variance documented at the Suregei tuff level therefore reflects disruption of patterns of individual ontogenetic development (this is graphically illustrated by plots of Raup's parameters⁵ W and T during ontogenesis—see Fig. 3). However, two major aspects of events at the Suregei tuff and Guomde levels are clearly at variance with Mayr's model: (1) the (obligatory) asexual taxon *M. tuberculata*³⁰⁻³³ shows a pattern of evolutionary change identical with other sexual species, at both the Suregei tuff and Guomde levels; and (2) evolutionary change at the Suregei level, although occurring rapidly, occurs over a large area and in thick faunal units containing many millions of individuals.

Both these observations question the significance of genetic drift, founder effect and inbreeding as mechanisms for triggering breakdown of homeostasis and speciation in geographical iso-

lates. Moreover, the events in the asexual taxon *Melanoides* question the conventional assumption that the significance of geographical isolation to speciation is blockage of gene flow (gene flow may in any case be an insignificant phenomenon, even in sexual species)³⁴. I propose elsewhere¹⁰ that geographical isolation is a prerequisite for speciation in that it shields transitional populations, vulnerable due to developmental instability, from competition with their unaltered ancestral taxa. The power of density-dependent stabilizing selection (the competitive vulnerability of phenodeviants to more 'modal' phenotypes), well documented by laboratory work³⁵ and by observations of natural populations³⁶, is relevant here.

Apart from the tantalizing insights into speciation mechanisms offered by the Turkana Basin sequence, it has two more general implications for evolutionary theory. The documented restriction of significant evolutionary change to speciation events indicates that the underlying unit of macroevolutionary change is the species. The fact that evolutionary change at the species level is shown to be punctuated and achieved by 'revolutionary' periods of extreme developmental instability strongly supports the notion that speciation is a qualitatively different phenomenon from gradual, intraspecific microevolutionary change^{29,37}.

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LETTERS TO NATURE

Rapid rotation of the solar interior

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The observation of line-of-sight velocities of the solar surface by means of Doppler shift measurements of the Fraunhofer absorption lines, using optical resonance scattering, has proved a useful technique in solar physics enabling the discovery of long period solar oscillation¹, the discrete, regular structure in the 5-min oscillation² and, as we report here the splitting of this structure by rotational effects. A measurement of the splitting of the discrete lines in the 5-min oscillations of the solar surface produced experimental evidence for the rapid internal rotation of the Sun. These data demonstrate that the Sun does not rotate uniformly but that the 'core' of the Sun rotates 2–9 times as rapidly as the observed surface rotation. The number of components into which the lines are split also allows unambiguous identification of 11 examples of each of the $l=0$, $l=1$ and $l=2$ modes in the frequency range 2.40–3.85 mHz.

Each phenomenon discovered by Doppler shift measurements has yielded unexpected information in view of the then current theoretical models of the Sun. The long period oscillation (160 m), also found³ using a different technique, is more than double the gravest radial mode predicted by linear oscillation theory and is still not understood. The global nature and large Q or long coherence time of the 5-min oscillations was contrary to what had, until demonstrated experimentally, been widely believed⁴, even after the work of Deubner *et al.*⁵.

The possibility of a Sun with a rapidly rotating core was suggested by doubts about the measurements of the rotation of the perihelion of Mercury, as a test of Einstein's general theory of relativity, due to the solar oblateness measurements of Dicke and Goldenberg⁶, and the existence of the scalar-tensor theories of gravitation⁷. Solar oblateness might imply a significant solar gravitational quadrupole moment which would contribute,

through purely newtonian effects, to the rotation of the perihelion of Mercury and thereby destroy the apparent agreement between general relativity and observation⁸.

Although the oblateness measurements could be interpreted in terms of a core of 0.56 solar radii with a uniform angular velocity 19 times that of the surface, another measurement indicated a spherical Sun⁹.

Other points of interest in rapid rotation are the distribution and transport of angular momentum in rotating fluids^{10,11}, the formation of planetary systems¹², the solar wind braking and the lithium and beryllium concentrations as a function of stellar age¹³. The problem is also relevant to theories of differential solar surface rotation^{14,15}, the dynamo theories of solar magnetism¹⁶ and the solar neutrino question¹⁷.

Ledoux¹⁸ demonstrated that oscillations of a uniformly rotating fluid body would be split into $(2l+1)$ components where l is the mode of the spherical harmonic. The resulting frequencies are given by

$$\nu = \nu_c \pm m(1 - C_{nl}) \frac{\Omega}{2\pi} \quad m = 0, 1, 2, \dots, l \quad (1)$$

where m is the azimuthal spherical harmonic index, C_{nl} is a positive constant ≤ 0.2 which depends on the equilibrium structure of the body and on the mode (n, l) considered, and Ω is the angular velocity.

Table 1 The central frequencies for lines in the range 2.40–3.85 mHz

n $\nu_{n,0}$ $\nu_{n,1}$ $\nu_{n,2}$ Mode		$\nu_{n,0}$	$\nu_{n,1}$	$\nu_{n,2}$
Radial overtone	(mHz)	(mHz)	(mHz)	
16		2.425	2.487	
17	2.496	2.559	2.620	
18	2.629	2.692	2.755	
19	2.763	2.829	2.888	
20	2.897	2.964	3.025	
21	3.033	3.099	3.160	
22	3.168	3.234	3.294	
23	3.303	3.370	3.430	
24	3.437	3.504	3.568	
25	3.576	3.640	3.704	
26	3.710	3.777	3.839	
27	3.848			

See text for tentative n value assignment.

Rotational splitting was verified experimentally for the Earth's normal modes of vibration excited by the Chilean earthquake¹⁹ and the possibility of observing it in the solar context was pointed out¹ and has been searched for in the case of high l value ($l \approx 200$) solar oscillations²⁰.

Thus, given sufficient resolution, the $l = 0$ mode should appear as a singlet, the $l = 1$ mode as a triplet, the $l = 2$ mode as a quintuplet and so on, each with a splitting of $0.4 \mu\text{Hz}$ if the Sun, rotating uniformly, were viewed from the Earth. However, when the full solar disk is observed, certain components may be reduced in amplitude or entirely absent, due to symmetries. Any greater observed splitting would indicate a more rapidly rotating solar interior, the exact value of which would depend on how the solar oscillation averaged these regions of differing angular velocity.

When considering a non-uniformly rotating body, Ω must be replaced by a suitably averaged angular velocity $\bar{\Omega}$. If the angular velocity is a slowly varying function of r only and large n and low l modes are considered:

$$\bar{\Omega} = \int_0^R \frac{\Omega(r)}{v(r)} dr / \int_0^R \frac{1}{v(r)} dr \quad (2)$$

where $v(r)$ is the velocity of sound²¹.

In addition to the rotational splitting, magnetic fields will cause asymmetries of the splitting²², however, fields of several megagauss are required to produce an observable effect.

An improved version of the Doppler shift optical resonance spectrometer²³ was used to record data on 28 contiguous days from 21 July to 17 August 1980 at the Teide Observatory on Tenerife. The basic measurements consisted of determinations of the resonantly scattered intensities corresponding to the two wings of the 769.9-nm Fraunhofer absorption line of neutral potassium. These data were recorded at 0.5-s intervals and subsequently transferred to digital magnetic tape together with timing information from a crystal clock. The mean line of sight velocity over a 42-s interval was then calculated from each day's data, and after subtraction of the velocities due to the Earth's rotation, orbital motion, the gravitational redshift, and other steady shifts¹, yielded a set of residuals for further analysis: 271 h of data were recorded giving 23,252 values, each with its appropriate time record. This data set was then subjected to an iterative sine wave fitting program with a frequency increment of $0.1 \mu\text{Hz}$, the frequency range covered was $2.40\text{--}3.85 \text{ mHz}$.

Because data are only recorded during daylight hours the data string has a 24-h amplitude modulation, which results in the appearance of side-bands displaced by $11.57 \mu\text{Hz}$ from the frequencies present in the data. This not only makes the calculated frequency spectrum difficult to visualize, but the side-bands may actually interfere with lines present in the data thus effectively reducing their apparent contribution. However, the line frequencies and their side-bands are related both in frequency and phase and thus the output from the sine wave fit, giving amplitude, frequency and phase, can be fully used to identify the lines present in the original data.

This procedure identified 33 lines which are listed in Table 1. Close examination showed that certain lines were split into triplets, others into quintuplets and another class was not split at all. Consequently these lines were identified as the $l = 1$, $l = 2$, and $l = 0$ modes of the spherical harmonic. Although the centroids of each line could be determined to $0.1 \mu\text{Hz}$, line pulling effects due to residual curvature in the data and the presence of noise limited the accuracy to an estimated $\pm 0.7 \mu\text{Hz}$ so that values are only quoted to the nearest μHz in Table 1. The radial overtone, n , cannot be directly determined, but from a

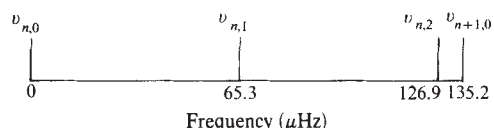


Fig. 1 The relative mean spacings of the central frequencies for the $l = 0, 1, 2$ lines in the frequency range $2.40\text{--}3.85 \text{ mHz}$.

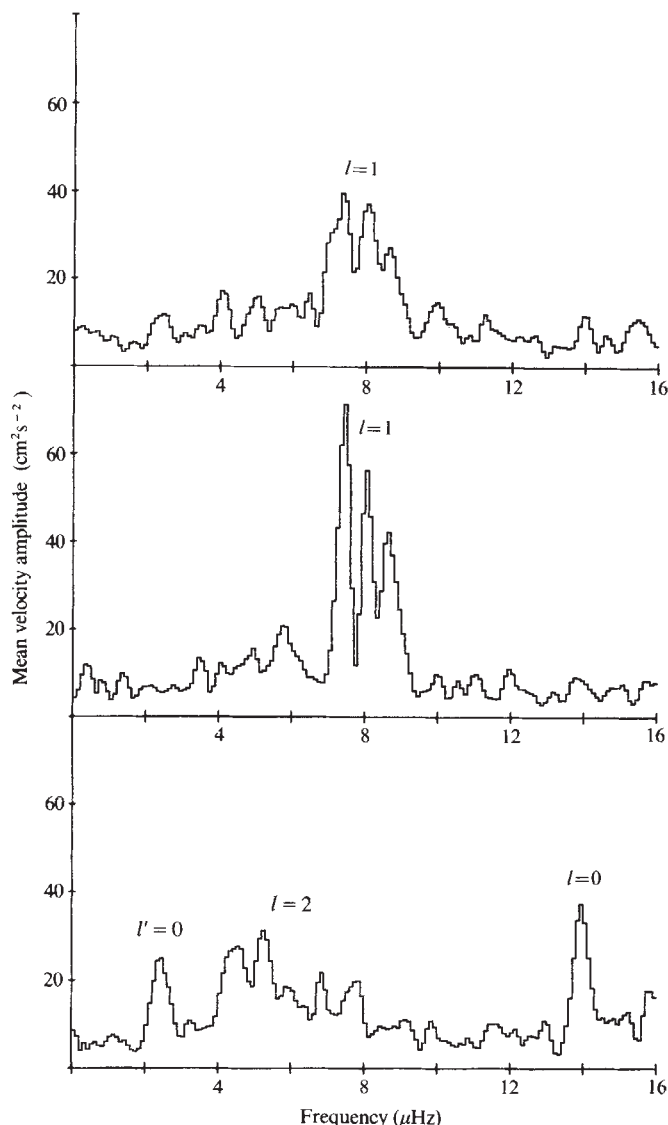


Fig. 2 Superposed frequency spectra for: a, the $l = 1$ line assuming a fixed frequency separation of $135.2 \mu\text{Hz}$; b, the $l = 1$ line based on the central component; c, the $l = 0$ and $l = 2$ lines based on the $l = 0$ line, where $l' = 0$ is the $l = 0$ side band displaced by $11.6 \mu\text{Hz}$.

comparison of the measured frequencies of the various identified modes with those calculated by Scuflaire *et al.*²⁴, values for n as shown in Table 1 were assigned.

These values agree well with those previously found²⁵ and the mean frequency separation of each mode as well as the inter-mode spacings are given in Table 2. The ordering of the various modes and their spacings, illustrated in Fig. 1, are in fair agreement with those recently determined by Grec *et al.*²⁶. Note that the errors quoted in Table 2 refer to the standard deviation of the mean of the 11 values found for each mode and thus the estimated standard deviation of a single frequency value of $0.7 \mu\text{Hz}$ seems justified if it is assumed that the frequency separations remain constant over this frequency interval. These values emphasize that, of the current generation of solar models^{27,28} only those with a heavy element abundance (Z) considerably below that of the standard solar model predict the observed frequency separations²⁹, yet a comparison of the actual frequencies would favour a more standard Z value. The calculations of Scuflaire *et al.*²⁴ based on a standard solar model with a convective zone of increased depth do, however, give the present best fit to both the frequency separation and the actual frequency values.

Table 2 The mean frequency separations of the various l modes in the range 2.4–3.85 mHz

$\nu_{n,0}-\nu_{n-1,0}$ (μHz)	$\nu_{n,1}-\nu_{n-1,1}$ (μHz)	$\nu_{n,2}-\nu_{n-1,2}$ (μHz)
135.2 ± 0.2	135.2 ± 0.1	135.4 ± 0.2
$\nu_{n,1}-\nu_{n,0}$ (μHz)	$\nu_{n,2}-\nu_{n,1}$ (μHz)	$\nu_{n+1,0}-\nu_{n,2}$ (μHz)
65.3 ± 0.4	61.6 ± 0.4	8.3 ± 0.3

A superimposed frequency spectrum for the $l = 1$ line assuming a mean spacing of $135.2 \mu\text{Hz}$ is shown in Fig. 2a. The triplet structure of this line is clearly evident, however, due to the line pulling effects, an improved spectrum is obtained if the superposition is based on the central component of each triplet as shown in Fig. 2b. A similar procedure based on the frequencies of the $l = 0$ line produces the spectrum in Fig. 2c. A single peak of width $0.5 \mu\text{Hz}$ is clearly resolved and the five peaks ($2l+1$) corresponding to the $l = 2$ mode are also evident. The effect of the 24-h modulation is illustrated by the $l' = 0$ side band displaced by $11.6 \mu\text{Hz}$ from the main $l = 0$ peak. The mean amplitude of the $l = 2$ frequency components is $\sim 6 \text{ cm s}^{-1}$ and hence the effect of noise is more evident due both to unfavourable observing conditions during the 1980 season and a possibly noisy source as the measurements were made during the solar maximum. The widths of the $l = 0$ and $l = 1$ peaks ($0.5 \mu\text{Hz}$) should be compared with the intrinsic resolution of the data set of $\sim 1/T$ ($0.43 \mu\text{Hz}$) where T is the length of the set in seconds. Hence the width observed is consistent with the intrinsic frequency resolution and thus indicative of high Q oscillations.

Assuming that all the splitting determinations have equal statistical weight yields a mean value of $0.75 \mu\text{Hz}$. This should be compared with the anticipated uniform rotation value of $0.4 \mu\text{Hz}$. The core of the Sun therefore indisputably rotates faster than the observed surface. Such a result eliminates some of the theories of differential rotations of the solar surface, as these require that the solar interior rotates more slowly than the surface¹⁵. If the radius of this high angular velocity core is almost that of the Sun then the core has approximately twice the surface angular velocity. The exact value will, however, depend on core radius, the depth of penetration of the p mode oscillation considered and the appropriate averaging of the effective angular velocity. As the radius of the core is decreased so its angular velocity increases. First-order calculations suggest that a core of 0.5 solar radii would, in terms of the observed splitting, have an angular velocity ~ 3 times that of the surface, and a core of 0.15 solar radii an angular velocity nine times that of the surface. These values fall far short of those required by Dicke¹³ and Roxburgh¹⁷ to explain the quadrupole moment deduced from the solar oblateness measurements and the low solar neutrino flux, respectively.

We thus tentatively conclude that the rotation of the interior of the Sun is rapid enough to be interesting in a solar and astrophysical context but appears far from rapid enough to contribute substantially to the quadrupole moment of the Sun.

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Pyroelectric luminescence

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We report here the observation of spontaneous light emission from molecular pyroelectric crystals which occurs when the crystal temperature is varied. We call this phenomenon pyroelectric luminescence (PEL). The intensity and time evolution of the light depends on several factors but is particularly sensitive to the pressure of air surrounding the crystal. This pressure dependence is similar for all the crystals and can be used to separate the luminescence into three classes. The distinguishing features of these classes may indicate the existence of different mechanisms.

A pyroelectric crystal is characterized by a unit cell with a net dipole moment, and, unlike a ferroelectric, the direction of the resulting lattice polarization cannot be changed by an external field¹. The spontaneous polarization of such crystals is compensated by the acquisition of free charges from the surrounding medium, by the internal conduction of free charges^{2,3} and by the migration of surface charges. Heating or cooling such a crystal induces a polarization until the charge distribution again reaches equilibrium. During this time, the magnitudes of the resulting electric fields may be large enough to produce dielectric breakdown in the ambient atmosphere and induce luminescence not only from the breakdown but also from the solid itself.

Observations of this luminescence were made by using a variable temperature cryostat fitted with a photomultiplier tube. This apparatus permitted control of the pressure in the sample chamber and allowed the sample temperature to be increased or

Table 1 Summary of observations

Compound	Pyroelectric	PEL
<i>N</i> -Acetylanthranilic acid	Yes	Yes
2,3-Dichloro-1,4-naphthaquinone	Yes	Yes
2,3-Dibromo-1,4-naphthaquinone	Yes	Yes
4,4'-Dibromobenzophenone	Yes	Yes
4,4'-Dibromodiphenylether	Yes	Yes
Phthalic anhydride	Yes	Yes
<i>m</i> -Bromonitrobenzene	Yes	Yes
Coumarin	Yes	Yes
Resorcinol	Yes	Yes
Acenaphthalene	Yes	Yes
4,4'-Dimethylbenzophenone*	No	No
Benzophenone*	No	No
Benzil*	No	No
Durene	No	No
1-Indanone	No	No
4,4'-Dichlorobenzophenone	No	No
Tetrachlorophthalic anhydride	No	No
Tetrabromophthalic anhydride	No	No

* Piezoelectric, but not pyroelectric, compounds.

decreased continuously at a selected rate. The crystals were freely mounted on the cold finger of the cryostat with a paper sleeve or in other cases with a small amount of silicon grease to ensure good thermal contact. The temperature of the cold finger was monitored with a Au/Fe-chromel thermocouple. Most of the compounds studied were obtained from commercial sources and were purified by recrystallization when the specified purity was <99%. Crystals were grown either from solution by evaporation or from the melt by the Bridgman technique.

Both pyroelectric and nonpyroelectric crystals were examined; the results are summarized in Table 1. Unlike the nonpyroelectrics, including compounds that are piezoelectric but not pyroelectric, all the pyroelectrics exhibited luminescence when the temperature was varied gradually. The intensity and the time evolution of the emitted light is a function of the rate of temperature change, the size of the crystal and the pressure of the atmosphere surrounding the crystal. The air pressure in the sample chamber dramatically alters the intensity and the time evolution of the luminescence and can be used to group the luminescence into three broad classes defined by the pressure ranging from 1 atmosphere to 1 torr (class 1), 1 torr to 1 mtorr (class 2), and <1 mtorr (class 3).

The overall luminescence intensity decreases as the air pressure in the sample chamber is reduced, and the effect of the reduced pressure on the time evolution is shown by Figs 1, 2. At the higher pressures, flashes of light are produced. In the case of coumarin, for example, these flashes have rise and decay times of <30 μ s. The observation of simultaneous current pulses in an external circuit connecting opposite faces of the crystal suggests that dielectric breakdown may be responsible for this luminescence. In the second pressure region, flashes still occur, but these flashes are preceded by rapid, less intense pulses. Note that after an intense flash, no light is emitted for some time, corresponding

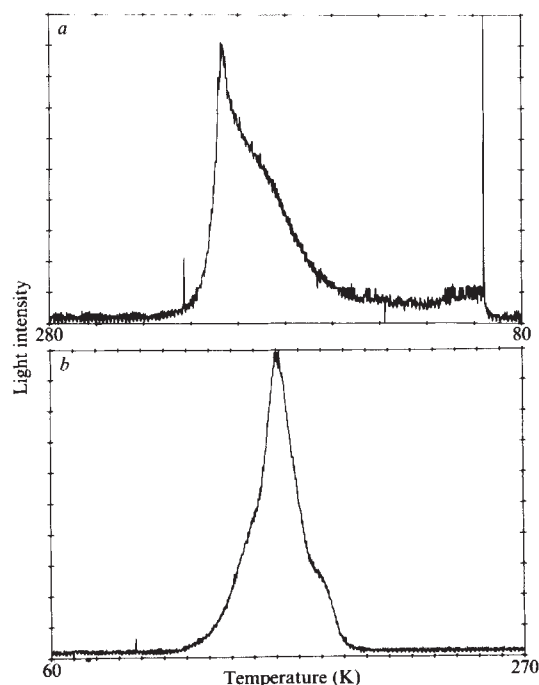


Fig. 2 *a*, Light intensity from a crystal of coumarin when cooled at the rate of $7^{\circ} \text{ min}^{-1}$ starting at 280K in class 3 conditions. *b*, Light intensity from a crystal of coumarin when heated at the rate of $2^{\circ} \text{ min}^{-1}$ starting at 60K in class 3 conditions. In both cases, the pressure of the air surrounding the crystal is ~ 0.05 mtorr.

to some change in temperature. Presumably during this time an electric field is building up to the threshold value needed to produce the luminescence. This sequence repeats itself almost periodically as the temperature gradually changes at a constant rate. In this class too, the luminescence is strongly correlated with current flow in the external circuit, but the presence of the rapid, less intense pulses indicates some difference in mechanism. In the third class the light intensity varies gradually with temperature and time, being observed only over a small temperature range and peaking at particular temperatures characteristic of the sample. It was thought that this behaviour might be caused by structural phase transitions, but for the case of coumarin, no evidence for a phase transition was found by comparing phonon Raman spectra at several temperatures.

While the dominant characteristics of the pressure dependence of the luminescence are described above, characteristics of one class may be found in the pressure region of another. For example, as can be seen in Fig. 1, class 2 luminescence contains some light flashes that are not preceded by less intense pulses. Also, the pressure limits of the classes should be considered only to define broad border regions.

The fact that only the pyroelectric crystals exhibit luminescence, in the conditions of the experiment, is evidence that the polarization field created by the temperature change is responsible for the light emission. We therefore use the term pyroelectric luminescence to describe this phenomenon. Further evidence that the mechanism responsible is electrical in nature is provided for classes 1 and 2 by the light pulses always being synchronous with current pulses in an external circuit across the sample. Dielectric breakdown of the ambient atmosphere is implicated for these classes by the fact that the intense flashes decrease in amplitude and frequency with decreasing pressure around the sample, although the less intense pulses that precede the intense flashes in class 2 may indicate an additional mechanism or at least some added complexity. The light emission for class 3 appears to be by a fundamentally different mechanism, perhaps involving excitation of the molecules through charge carrier recombination. Further evidence for the mechanisms involved may be obtained by measuring the spectral distribution of the luminescence.

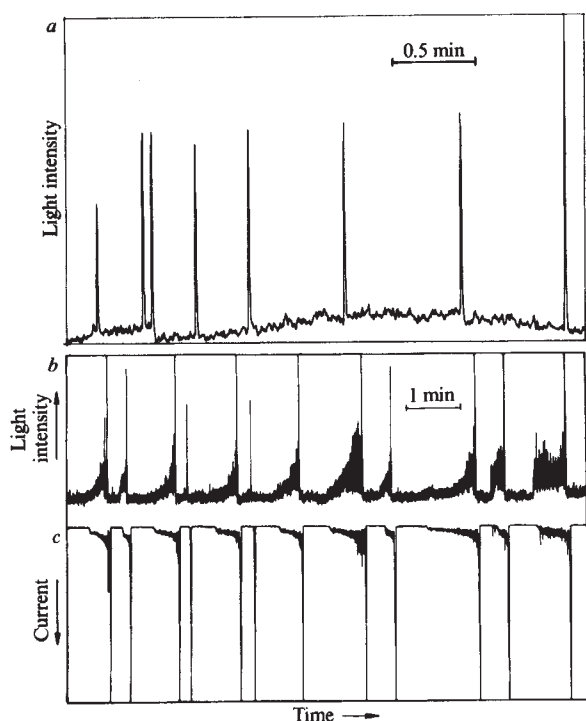


Fig. 1 *a*, Light intensity from a crystal of coumarin when cooled at the rate of $2.7^{\circ} \text{ min}^{-1}$ starting at 290K in class 1 conditions, the pressure of the air surrounding the crystal being ~ 760 torr. Rapid, less intense pulses do not appear even with higher amplifier gain. A similar pattern is observed when the crystal is heated. *b*, Light intensity from a crystal of coumarin when cooled at the rate of $5^{\circ} \text{ min}^{-1}$ starting at 270K in class 2 conditions, the pressure of the air surrounding the crystal being ~ 1 mtorr. The more intense flashes saturate the amplifier. Similar behaviour is observed when the sample is heated. *c*, Simultaneous measurement of the current flow in an external circuit connecting opposite faces of the crystal.

Pyroelectric luminescence is similar to and distinct from other luminescent phenomena. Like thermoluminescence⁴, light is emitted when the crystal is heated, but unlike thermoluminescence, light also is emitted when the crystal is cooled. Like electroluminescence⁵, the light in class 3 conditions may result from charge carrier recombination, but unlike electroluminescence, charge carrier injection by an external circuit is not needed. For triboluminescence, the light results from the application of a mechanical stress^{6,7}. Although thermal shocks have been used to produce triboluminescence, we have tried to avoid this possibility. The crystals were mounted freely to allow for expansion and contraction, and the temperature was changed gradually. The light in triboluminescence has been associated with the formation of cracks^{8,9}. Although cracks may contribute to triboluminescence in pyroelectrics, we see light emission but do not see cracks develop in the crystals studied even with repeated temperature cycles. Also, the slow variation of the light intensity with temperature and time in class 3 conditions, see Fig. 2, does not resemble that found for triboluminescence^{8,9}. Finally, crystals of benzil which are triboluminescent¹⁰, but belong to a nonpyroelectric space group¹¹, did not exhibit luminescence when heated or cooled gradually in our experiments.

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Theory of the dielectric constant of ice

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The dipole moment of a water molecule in liquid water differs from that of the isolated molecule because each molecule is further polarized by the electric field of the surrounding molecules. The effective dipole moment has to be known before a satisfactory statistical mechanical study of water and aqueous solutions is possible. One approach is to study ice rather than water because it has a known, simple structure. However, the recent literature on the theory of the dielectric constant of ice is confused and contradictory; for example, there are two rival theoretical expressions for the dielectric constant in terms of the dipole moment. A simple resolution of this 'internal field' problem is proposed here. It is further shown that the Bernal–Fowler–Pauling ice-rules model of ice behaves like a dielectric material, and matching the results of calculations on this model to experiment gives a dipole moment of 3.0 D.

The Bernal–Fowler–Pauling ice rules^{1,2} specify that each oxygen is bonded to two of the four hydrogens with which it is coordinated and that there is one hydrogen between each oxygen and its four nearest oxygens. Each hydrogen is bonded to one oxygen and 'hydrogen bonded' to another. With each oxygen fixed at a lattice site there remain, in most forms of ice,

many different possible arrangements of the hydrogens, and this quantitatively explains^{2,3} the observed residual entropy of ice, $\Delta S \approx R \ln 3/2$, as the contribution of the disorder in the hydrogen positions to the total entropy. If Bernal–Fowler–Pauling ice were also a good model for the dielectric properties of real ice, then comparison with experiment would give the effective dipole moment of a water molecule within the ice.

Nagle⁴ has suggested that real ice is very different from perfect, ice-rules ice because it contains a very small number of infringements to the ice rules, which make a major contribution to the dielectric properties and that neither of the rival equations for the dielectric constant properly applies in this case. Here, it is argued that both rival equations are correct but apply in different situations. It is assumed that while real ice does indeed contain defects these have little or no bearing on the static dielectric properties, though they are essential in the mechanism of dielectric relaxation⁵. That is, the low concentration of defects affects only the rate at which ice responds to an applied field but not the final polarization.

By considering a spherical volume containing N dipoles surrounded by an infinite dielectric continuum of dielectric constant ϵ' , and applying the laws of electrostatics and statistical mechanics⁶, the general expression

$$y \frac{\langle \mu_1 \cdot \sum_{i=1}^N \mu_i \rangle_{\epsilon'}}{\mu^2} = \frac{(\epsilon - 1)(2\epsilon' + 1)}{3(\epsilon + 2\epsilon')} \quad (1)$$

where

$$y = N\mu^2/9\epsilon_0 kTV \quad (2)$$

may be derived. μ_i is dipole moment of molecule i , ϵ_0 the permittivity of free space, k Boltzmann's constant, T the absolute temperature and V the volume. The simplification has been made that the dipoles are rigid and non-polarizable so that $\epsilon_\infty = 1$, this does not affect the essential physics. The derivation assumes that the system of dipoles constitutes an homogeneous and isotropic dielectric with dielectric constant ϵ , determined by the average response to an arbitrarily small applied electric field. The equation is independent of the form of the dipole–dipole and other interactions within the system. It is assumed that an applied field alters only the electrostatic interaction between the dipoles and the surrounding continuum, and this approximation becomes exact in the limit $N \rightarrow \infty$.

In the special case that the system is surrounded by a medium of the same dielectric constant as itself, $\epsilon' = \epsilon$, then the Kirkwood^{6–8} equation results. Taking the limit $N \rightarrow \infty$:

$$yg_k = \frac{(\epsilon - 1)(2\epsilon + 1)}{9\epsilon} \quad (3)$$

where the Kirkwood g -factor is

$$g_k = \lim_{R \rightarrow \infty} \lim_{r \rightarrow \infty} \left\langle \mu_1 \cdot \sum_{r_1 < r} \mu_i \right\rangle / \mu^2 \quad (4)$$

and R is the radius of the larger sphere of dielectric continuum. Another useful equation results by letting $\epsilon' \rightarrow \infty$ in equation (1). In the limit $N \rightarrow \infty$:

$$\epsilon - 1 = 3yG \quad (5)$$

where

$$G = \lim_{N \rightarrow \infty} \left\langle \mu_1 \cdot \sum_{i=1}^N \mu_i \right\rangle / \mu^2 \quad (6)$$

Equation (5) can also be derived by considering a slab of material between the plates of a condenser; it corresponds to the 'tin-foil' case where the system is surrounded by a perfect conductor which cancels all induced surface charges. Thus equation (5) applies not only for spherical geometry but in many other cases, including the periodically repeating cubes used in computer simulation^{9,10}.

If the system of dipoles obeys classical electrostatics and has a meaningful dielectric constant then equation (1) is always correct and $\langle \mu_1 \cdot \sum_{i=1}^N \mu_i \rangle_{\epsilon'}$ must be a function of ϵ' such that ϵ is

invariant to ϵ' in the infinite system limit. Either the system does not have a dielectric constant or both equations (3) and (5) are correct but correspond to physically different situations. These are the two 'rival' equations for the dielectric constant of ice. Nagle⁴ argues that in most cases, including real ice, one or both of equations (3) or (5) do not apply and that $G = g_k$. It has been argued¹¹⁻¹³, especially by Stillinger and Cotter¹⁵, that $G > g_k$ for the special case of ice-rules ice. Both they and Nagle⁴ argued that permitting an arbitrarily small but non-zero concentration of defects to the ice rules would reduce G to g_k in the infinite system limit. However, real ice has long-ranged electrostatic interactions between the dipoles of the water molecules and these force $G > g_k$ such that both equations (3) and (5) hold. The theory proposed here is that the correlations found in the perfect ice-rules model are to a good approximation the same as those that long-range dipole-dipole interactions will produce.

The Bernal-Fowler-Pauling ice rules specify a potential energy which is zero for a permitted configuration and plus infinity for one which breaks the ice rules. There are no terms corresponding either to the high energy of an isolated system with a large net dipole moment or to interactions with a surrounding medium. The ice rules can, therefore, correspond to only one situation: the model is that of a block of perfect ice surrounded by a perfect conductor. Thus $\langle \mu_1 \cdot \sum_{i=1}^N \mu_i \rangle / \mu^2$, where N is the number of dipoles, is G ; and calculations claiming^{14,16} to have calculated g_k have in fact calculated G , a fact that has been recognized before^{11,15}.

Rahman and Stillinger¹⁷ estimated g_k using a Monte Carlo method. They simulated a periodically repeating block of ice obeying the ice rules with the additional requirement that $\sum_{i=1}^N \mu_i = 0$. They estimated g_k by summing over all dipoles within a spherical region around each dipole taken in turn. In the limit that the sphere radius is infinitely large but infinitely smaller than the size of the periodic block this will give the true value of g_k . For ice I_c they used a cube of 4,096 water molecules and found a g_k of 1.84. A simple correction for the finite size produced a final estimate of $g_k = 2.11 \pm 0.10$. A similar value was obtained for ice I_h .

A simpler and more accurate Monte Carlo calculation¹³ can be made in much the same way as Rahman and Stillinger by removing the requirement that $\sum_{i=1}^N \mu_i = 0$. Then an estimate for G can be found from

$$G = \left\langle \left(\sum_{i=1}^N \mu_i \right)^2 \right\rangle / N\mu^2 \quad (7)$$

whether the system of N water molecules has periodic boundary conditions or not. New Monte Carlo calculations have been made to calculate G in this way for cubic ice, ice I_c , and the results are given in Table 1. When N is small then not all long-range correlations in dipole orientations are included and

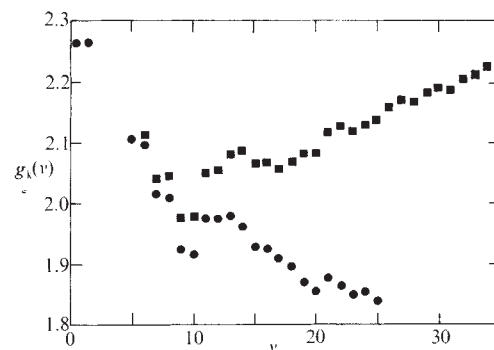


Fig. 1 ●, $g_k(v)$ from ref. 17, in which the cube moment of fluctuations were suppressed so that $g_k(v)$ goes to zero when taken over the entire cube of nearest images. ■, $g_k(v)$ from the present work, for which at large v $g_k(v)$ rises almost monotonically to G when taken out to the cube of nearest images. Points are omitted where they would overlap with the ref. 17 data points. v is the number of the coordination shell. For full details see ref. 17.

G is underestimated. When N is large then only a small fraction of the random loops are 'open' to effect a change in $\sum_{i=1}^N \mu_i$ and so the statistics are poor. The present result that $G = 3.04$ is in good agreement with previous calculations using other methods^{11,16,18}.

g_k may be decomposed into a function of the number of coordination shells contributing to it, so

$$\left. \begin{aligned} g_k(v) &= g_k(v-1) + \sum_{i \in v} \mu_i \cdot \mu_i \\ g_k(0) &= 1 \end{aligned} \right\} \quad (8)$$

where the sum is over all dipoles in the v th coordination shell. The true g_k is $g_k(\infty)$ which is not attainable from computer simulation. Figure 1 compares $g_k(v)$ from the present work with that found by Rahman and Stillinger. Both calculations used $N = 4,096$ and allowing for the size of the systems g_k is indeed close to 2.0. The ratio $G/g_k \approx 3/2$ is exactly what is required for a model of a material with $\epsilon \gg 1$ and ice-rules ice therefore behaves like a dielectric. The model does not exactly obey the laws of electrostatics, because the ice rules determine G/g_k to be independent of y , whereas electrostatics requires

$$G/g_k = 3/2(3yG + 1)/(3yG + 3/2) \quad (9)$$

which is independent of y only as $y \rightarrow \infty$. However, if $3yG$ is large, and for ice I_h it is of the order of 100, then the model is quite satisfactory.

Thus g_k for ices I_c and I_h is close to 2.0 and the corresponding estimate for the effective dipole moment of a water molecule in ice is 3.0 D, a large increase on the isolated molecule moment of 1.85 D (ref. 19). However, the model assumes all possible arrangements to be of equal energy, which is only approximately true^{14,17}. In simple polar liquids it is found that the dipole-dipole interactions alone are sufficient to raise g_k considerably as y is increased¹⁰. Such interactions are present in ice also and may raise both G and g_k by producing weak long-range correlations in addition to those imposed by the ice rules. These dipole-dipole interactions would also make G/g_k a function of y which should then obey equation (9). These correlations should be distinguished from ordering in the crystallographic sense, which would reduce ϵ . The Bernal-Fowler-Pauling ice-rules ice gives a good approximation to the static dielectric properties of real ice, but one which almost certainly underestimates the correlations which contribute to the high dielectric constant of real ice.

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Table 1 Monte Carlo results for G for a series of cubes of ice I_c with periodic boundary conditions

N	G	Error limit	No. random loops/ 10^5
8	2.667	± 0.015	2
64	2.938	± 0.02	2
216	2.980	± 0.02	5
512	2.986	± 0.06	5
1,000	3.005	± 0.09	5
1,728	3.044	± 0.12	6.6
2,744	3.044	± 0.07	16.7
4,096	3.027	± 0.26	20

The error limits were given by the maximum difference between $3\langle (\sum_{i=1}^N \mu_{xi})^2 \rangle$, $3\langle (\sum_{i=1}^N \mu_{yi})^2 \rangle$ or $3\langle (\sum_{i=1}^N \mu_{zi})^2 \rangle$ and $\langle (\sum_{i=1}^N \mu_i)^2 \rangle$. For $N = 8$ it would be feasible to obtain G exactly because the number of possible configurations is small but this has not been attempted. In all cases the estimated squared mean polarization, $\langle (\sum_{i=1}^N \mu_i)^2 \rangle$, which must go to zero in an infinitely long calculation, was negligible compared with $\langle (\sum_{i=1}^N \mu_i)^2 \rangle$.

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Amorphous structure of metamict minerals observed by TEM

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Metamict minerals are a special class of materials which were initially crystalline but have become amorphous because of accumulated structural damage caused by the radioactive decay of their constituent U and Th nuclides^{1–3}. Damage from α particles and recoil nuclei may cause dramatic changes in physical, chemical, mechanical and structural properties of materials^{4–6}. Thus, metamict minerals provide a natural example of the potential long-term effects of radiation damage in proposed crystalline radioactive waste forms⁷, such as the Sandia ceramic waste forms^{8–10}, SYNROC^{11–13}, super-calcine¹⁴ or the tailored ceramics¹⁵. The determination of the microstructures of metamict materials, therefore, may help to predict long-term radiation effects in crystalline waste forms. This report presents the results of an examination by transmission electron microscopy (TEM) of a wide range of metamict silicate and complex Nb-Ta-Ti oxide minerals.

Most previous studies of metamict minerals have been restricted to detailed X-ray diffraction analyses or measurements of changes in physical and optical properties with increasing α dose^{16–17}. None of these techniques allows the structure of the metamict state to be interpreted at the near-atomic level. Until recently there have been only cursory studies utilizing the TEM. Christ *et al.*¹⁸ report sharp electron-diffraction patterns of metamict samarskite, polyclase and

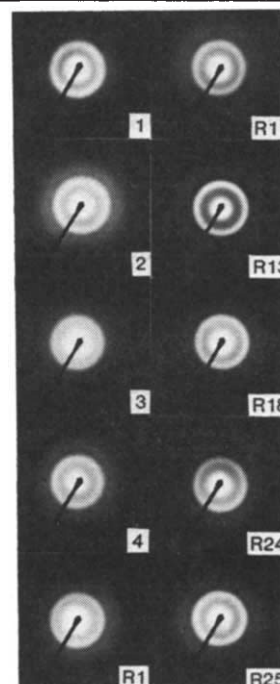


Fig. 1 Selected area diffraction pattern of metamict: 1, allanite; 2, gadolinite; 3, yttrialite; 4, thorite; R1, aeschynite; R11, priorite; R13, blomstrandine; R18, euxenite; R24, blomstrandine; R25, euxenite.

zircon. Bursill and McLaren¹⁹ report sharp electron diffraction patterns for metamict zircon (amorphous based on X-ray diffraction, with an α dose $>10^{16}$ mg^{-1}) which they interpreted as resulting from slightly misoriented zircon crystallites ~ 100 Å in size. Sommerauer²⁰ reports both crystalline and amorphous areas in metamict zircons. We have used TEM to study crystalline, partially metamict, and fully metamict zircons²¹. The fully metamict zircon possessed two distinct structures, one interpreted as amorphous and the other as being composed of slightly misoriented microcrystallites of 50–100 Å size with tilts of up to 10° . The microcrystallites were interpreted as later alteration and recrystallization features, unrelated to the process of metamictization. Yada *et al.*²² have applied high resolution electron microscopy in a study of the metamictization process in zircon and confirmed that the metamict state in that material is completely amorphous.

In the present study, a wide range of metamict minerals, including silicates and oxides, was examined by TEM to

Table 1 Metamict minerals examined in this study

Sample no.	Museum no.	Mineral	Ideal formulae*	Locality	Ref.
Silicates:					
1	—	Allanite	$(\text{Ce,Ca,Y})_2(\text{Al,Fe})_3(\text{SiO}_4)_3(\text{OH})$	Rode Ranch, Texas, USA	24†
2	—	Gadolinite	$\text{Be}_2\text{FeY}_2\text{Si}_2\text{O}_{10}$	Rode Ranch, Texas, USA	24, 25
3	—	Yttrialite	$(\text{Y,Th})_2\text{Si}_2\text{O}_7$	Rode Ranch, Texas, USA	26
4	—	Thorite	ThSiO_4	Cardiff, Ontario, Canada	
Nb-Ta-Ti oxides:					
R1	Stanford University 473	Aeschynite	$(\text{Ce,Ca,Fe,Th})(\text{Ti,Nb})_2(\text{O,OH})_6$	Hitterö, Norway	6, 16, 27, 29
R11	C. O. Hutton collection	Priorite	$(\text{Y,Ca,Fe,Th})(\text{Ti,Nb})_2(\text{O,OH})_6$	Ahi-Trombe, Madagascar	27
R13	Harvard 84647	Blomstrandine	$(\text{Y,Ca,Fe,Th})(\text{Ti,Nb})_2(\text{O,OH})_6$	Morefjaer, Norway	16, 27
R18	Harvard 89653	Euxenite	$(\text{Y,Ca,Ce,U,Th})(\text{Nb,Ta,Ti})_2\text{O}_6$	Betsiboka Valley, Madagascar	6, 27
R24	Mineralogisk-Geologisk Museum (Oslo)	Blomstrandine	$(\text{Y,Ca,Fe,Th})(\text{Ti,Nb})_2(\text{O,OH})_6$	Kåbuland, Iveland, Norway	16, 27, 28
R25	Mineralogisk-Geologisk Museum (Oslo)	Euxenite	$(\text{Y,Ca,Ce,U,Th})(\text{Nb,Ta,Ti})_2\text{O}_6$	Eitland, Farsund, Norway	16, 27, 28

* For complete chemical analyses see the cited refs.

† A. J. Ehlmann, personal communication.

determine whether the metamict state is truly amorphous or consists of microcrystalline domains. The 10 samples used were carefully selected so that altered areas could be minimized. The mineralogical descriptions of the selected samples (see Table 1) including complete chemical analyses, X-ray diffraction analyses, annealing studies, differential thermal analyses and determinations of physical properties are given elsewhere.

Specimens for electron microscopy were prepared by grinding under a drop of butanol in an agate mortar and pestle and collecting the thin flakes on holey carbon substrates. Regions overlapping holes in the substrate were suitable for high resolution microscopy. The specimens were examined at 200 kV in a JEM-200CX electron microscope equipped with a high resolution (1.4 Å line, 3.5 Å point-to-point) objective lens pole piece. Electron diffraction, high resolution dark field imaging, and interference image microscopy were used to characterize the metamict structure (see ref. 23).

A minor amount (~10% maximum) of clearly crystalline material was observed in all 10 samples. Such regions were interpreted as later altered material, similar to altered crystalline regions observed in metamict zircon²¹, and are not considered further here. Electron diffraction patterns from the remaining material in all samples contained one or two broad haloes characteristic of 'amorphous' materials. Figure 1 shows the striking similarity between the patterns of the 10 samples. High resolution dark field imaging was used to investigate whether such patterns could be produced by diffraction from very small, randomly-oriented microcrystallites instead of amorphous structures. The images, obtained with the objective aperture positioned on an arc of the first broad diffraction ring, contained bright coherently scattering spots ~6–8 Å in size, see Fig. 2. This observation eliminates the possibility that the metamict structures consist of microcrystallites of 50–100 Å size because such crystallites would have been readily resolved as bright spots of that size. The interpretation of bright spots in the <10 Å size range is not straightforward, however, and their occurrence has been used as evidence for both microcrystalline and random network models of the amorphous state (see ref. 23). Interference (lattice) image microscopy in both the tilted and defocused axial illumination modes was then used to examine for small regions of fringes indicative of microcrystallites. Fringe-like contrast was not observed in either mode, apart from occasional random alignment, so this technique did not provide further evidence for the presence of

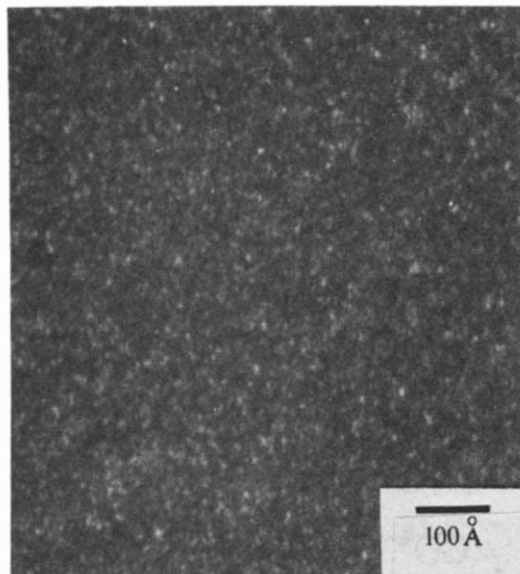


Fig. 2 Dark field image from a thin edge of Rode Ranch gadolinite (no. 2) with part of the first broad diffraction ring included within the aperture. Bright spots are ~6–8 Å in diameter.

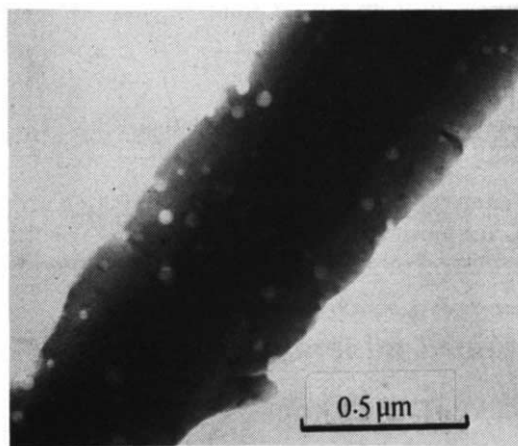


Fig. 3 Spherical microvoids in a thin chip of metamict euxenite (R25).

microcrystalline domains. Note that microcrystallites in the 50-Å size range would have produced similar-sized regions of fringes readily detected by the interference imaging technique. We concluded that the metamict state in these samples is amorphous in the sense that, if microcrystallites were present in these structures, they must be <~10 Å in size.

One important feature, common to the microstructure of all the complex Nb-Ta-Ti oxides, was the widespread occurrence of spherical microvoids (Fig. 3). We propose that those voids are generated by helium buildup from the internal α particle decay. Microvoids were not found in any of the silicates suggesting either that helium diffuses relatively rapidly through the silicate structures, or that helium is somehow accommodated in the structure.

This study shows that the metamict state in a wide variety of silicates and complex Nb-Ta-Ti oxides is amorphous to within the resolution capabilities of current electron microscope techniques (~10 Å). Void formation accompanies metamictization in the complex Nb-Ta-Ti oxides, but this does not occur in the silicates.

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Deep seismic reflection survey across the Variscan Front of southern England

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In 1979, the Institute of Geological Sciences commissioned two lines of seismic reflection profile, a technique which has proved successful¹ for studying crystalline basement. The survey was sited to cross the concealed Variscan Front in southern England, with recording time extended to 12 s two-way time (TWT). Similar profiles in adjacent parts of Europe have suggested that the Variscan Front is characterized by major thrust zones extending to considerable depths^{2,3}. Our profiles, reported here, show good primary reflections to 6 s TWT and give new detail on the nature of Variscan deformation in southern England. Zones of differing reflection character are evident; a northern zone of low northerly apparent dips, a central zone of poor reflector quality, and a southern zone of steeper dips to the SSE. The southern zone is presumed to represent deformed Variscan basement, including several minor thrust planes and a proposed major thrust zone, analogous to similar structures in Europe. Impersistent reflections between 9 and 10 s TWT could possibly relate to the Moho.

Two reflection profiles were recorded, totalling some 49 km (Fig. 1), Line 79-01 extending 30 km N from Devizes and line 79-02 extending 19 km E from its intersection with 79-01. The N-S profile was shot predominantly over Jurassic clays, while the E-W profile extended on to the Cretaceous outcrop. The seismic data were recorded using routine oil industry parameters: 4.5 kg explosive charges at 18 m depth in clay, 9 kg charges at 25 m depth in chalk, with shotpoints at 100-m

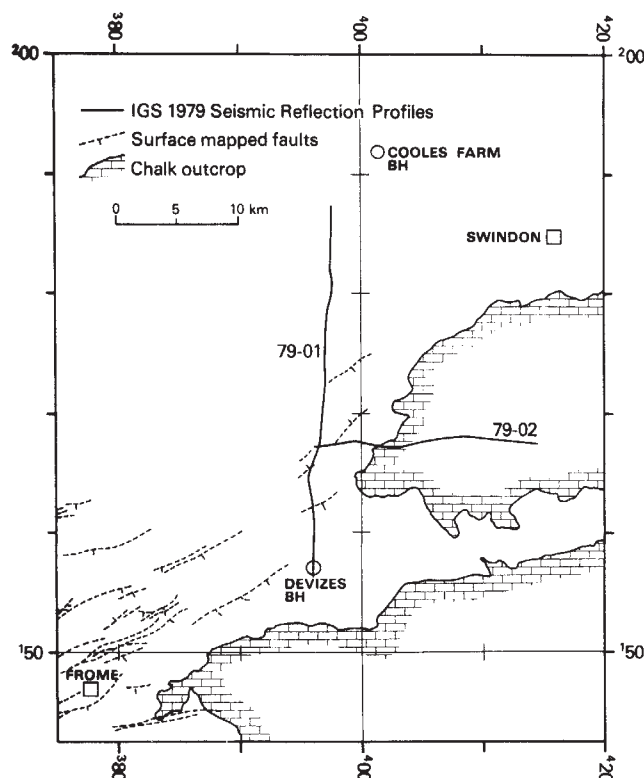


Fig. 1 Location map.

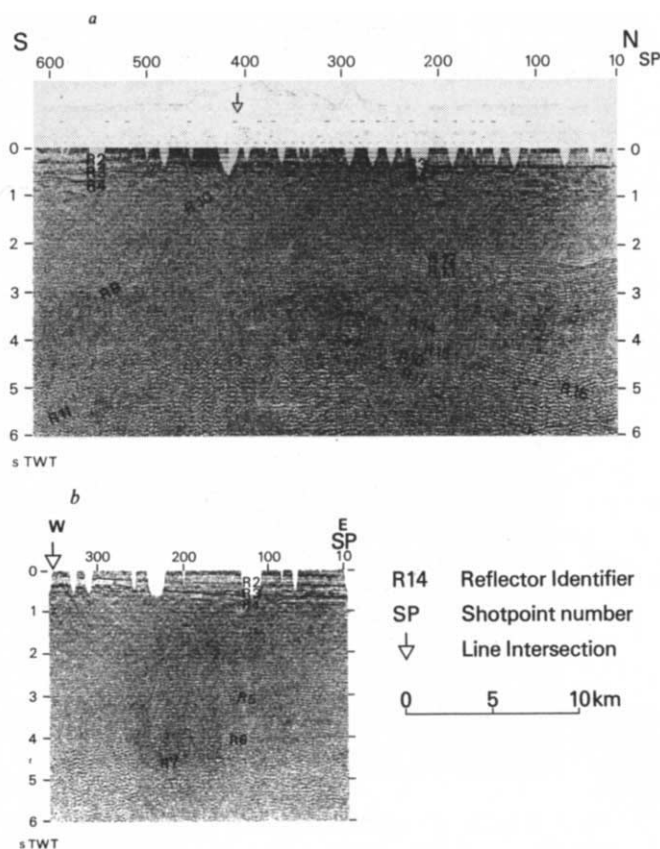
Geological Survey maps show several ENE-trending faults in the area, displacing Jurassic strata. Towards Frome, south-west of Devizes, these faults parallel the Mendip Variscan structures. Further geological control is available from the Devizes No. 1 Borehole⁴, which penetrated Jurassic, Triassic and Lower Carboniferous strata, and terminated in Devonian rocks; the total depth reached was 1,014 m, equivalent to ~0.7 s TWT. The

Table 1 Seismic data

Unit	Seismic interval	Density (g cm ⁻³)	P-wave seismic velocity (km s ⁻¹)
1	Surface to R3	2.35	2.6
2	R3-R4	2.40	3.2
3	R4-R12	2.50	4.0 (S) 3.9 (N)
4	R12-R17	2.65	5.5
5	Below R11 and R17	2.78	6.5

intervals, recorded by a 48-channel splitspread array, at a 50-m station interval. This configuration resulted in 12-fold of subsurface cover. A conventional processing sequence, at 4 ms sampling rate, which included automatic residual statics correction, was then applied to produce the final sections (Fig. 2).

Velocity analyses used in the stacking process show interval velocities rising sharply around 2.3 s TWT, to unrealistically high values. At depth, such high velocities do not affect the record quality, but depth conversion based on these data would be implausible. The velocity profile for the section, therefore, is based on a combination of borehole data, section interval velocities, and, below 2 s TWT, on values which were limited to a more reasonable maximum of 6.5 km s⁻¹ (see Table 1).



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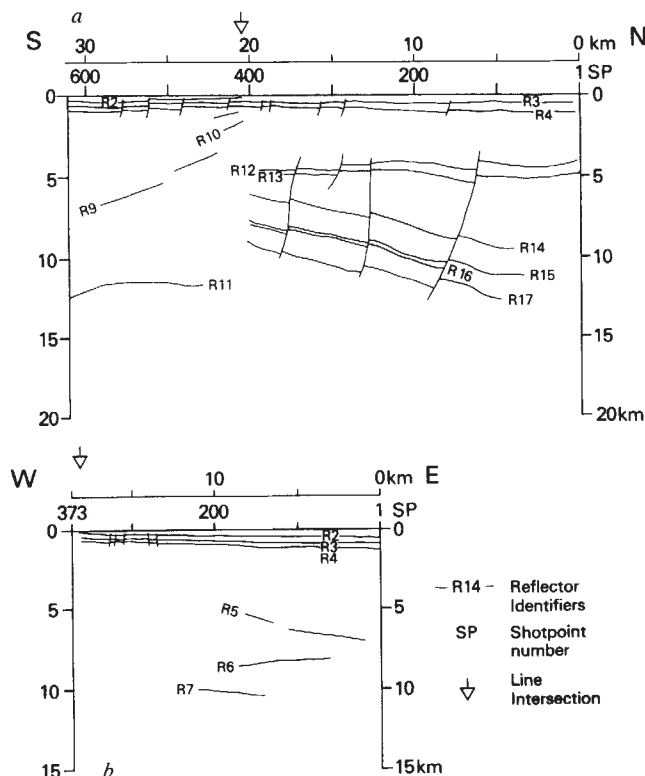


Fig. 3 Migrated depth sections. *a*, Line 79-01. *b*, Line 79-02.

northern end of the line can reasonably be extrapolated 7 km NE, and correlated with the Cooles Farm Borehole⁵.

Using both unmigrated and migrated data, the most significant primary events which we recognize are identified in Figs 2 and 3. Reflectors R2 to R4 are related to the Jurassic and Permo-Triassic succession, and are seen to be affected by minor faulting which can be correlated with surface geology. The northern half of line 79-01 is characterized by a strong reflector R12, with a significant rise in interval velocity across the interface. This is attributed to a distinct lithological boundary, and correlated with the top of the Cambrian quartzite proved in the Cooles Farm Borehole. Beneath R12 and R13, a complex series of reflectors occurs, the base of this unit being recognized as R17. This suite of reflectors exhibits an apparent dip of up to 20° to the north, with several significant faults, showing a calculated

dip-slip component of movement of up to 500 m. Although no positive geological identification can be established, the interval R12-R17 has a reflection style interpreted as being characteristic of a succession of sedimentary or volcanic rocks of possible Longmyndian and/or Uriconian affinity. This, combined with their depth relative to the Cooles Farm Borehole, suggests a Lower Cambrian to Proterozoic age for this seismic interval. This succession overlies, with angular discordance, a series of reflectors, which are presumed to derive from impedance contrasts within more highly metamorphosed Precambrian rocks.

A zone of very poor reflection quality separates the above northerly part of the section from that south of the intersection with line 79-02. Here, prominent reflectors have southerly apparent dip, and in the case of R9 and R10 the dips are significantly steeper than those to the north. Between 9 and 10 s TWT, several sub-horizontal events have been tentatively identified. These may relate to the Moho at around 30 km depth. On line 79-02 few coherent deep reflectors are identifiable below the sequence R2-R4, except in the east half of the line, where reflectors R5-R7 have been picked, R5 and R7 showing apparent dips to the east. Beneath R4 there seems to be, therefore, a lateral change in record character near the intersection of the two lines, indicated by a zone of poor reflection quality and discontinuous reflector segments.

Following migration and depth conversion, true scale depth sections were constructed (Fig. 3). The southern zone of line 79-01 contains reflectors R9 and R10 which are the most steeply dipping observed in the area. The nature of R9 is uncertain; a correlation with R12 is possible, but unlikely for two reasons.

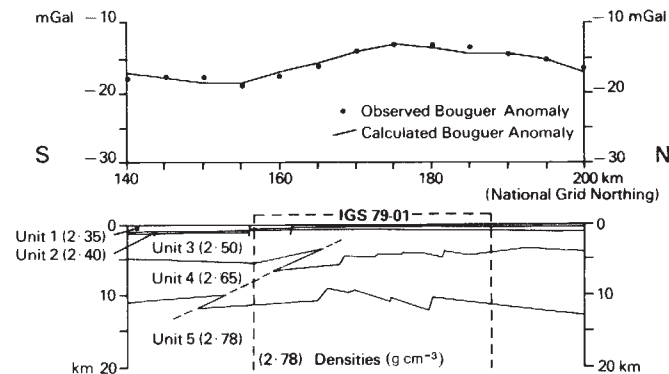


Fig. 5 Gravity profile including line of section.

First, the reflector sequence below R12 is quite unlike that below R9, and second, if the density contrast known to exist across R12 were to be present across R9, a negative gravity anomaly of at least 10 MGal would be expected, and this is not observed. It is possible that R9 represents a thin stratigraphical unit with no associated density increase, but its linearity and lateral persistence lead us to conclude that R9 represents a major structural interface. Some 25 km to the west, in the Mendip Hills, Variscan basement comes to outcrop. The structure is complex with folds and associated thrusts mapped at the surface⁶. One major thrust belt, the Southern Overthrust, lies ~8 km to the north of a folded inlier of Silurian volcanic rocks. These give rise to a prominent aeromagnetic anomaly, which continues ENE to intersect line 79-01 some 6 km S of the reflector R10, and probably defines the Variscan structural trend in the area⁷. The apparent dips of R10 are not dissimilar to that of the Southern Overthrust, which has a true dip of 21° to the SSE. It is reasonable to conclude therefore, that the reflectors R10 represent thrust planes of similar geometry to those observed in the Mendips. R9 has a similar aspect to R10, and is interpreted as a major thrust within the same tectonic regime. When correlated with the dipping event R5 on line 79-02, the resulting surface (Fig. 4) has a strike parallel to the probable local Variscan structural trend and a dip similar to the thrusts described above. The presence of a major thrust plane in

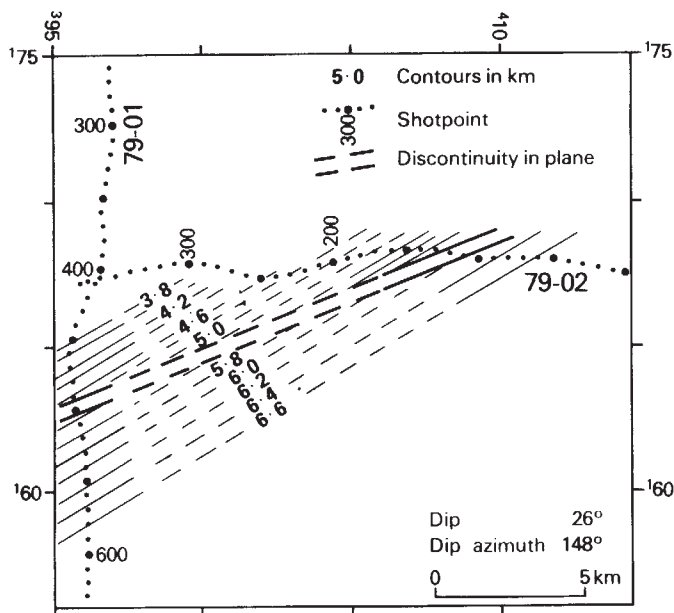


Fig. 4 Structure on proposed thrust plane.

the area is consistent with the observed Bouguer gravity field⁸. Figure 5 shows a N-S two-dimensional model based on this interpretation.

The survey allows the first observation of the deeply buried British Variscides, and provides a geometric model of structure to a depth of at least 10 km. The proposed major thrust plane may be tectonically analogous to structures delineated by similar methods over the Variscan Front in Europe. The survey demonstrates that standard oil company acquisition techniques, using an explosive energy source, with extended recording time, can provide deep reflection data of high quality. With industry cooperation, an inexpensive method of acquiring potentially useful deep seismic reflection data has now been demonstrated.

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Stable isotopes in a branching coral monitor seasonal temperature variation

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Reef corals, both fossil and recent, are potentially useful monitors of the surface ocean 'climate'. Because coral reefs flourish only in relatively warm water^{1,2}, the very presence of reef corals constrains the estimation of local temperature ranges. Studies of hermatypic corals have shown that coral growth rate^{3–8}, density band formation (in some species)^{9–15}, and stable isotopic composition^{14–23}, all respond to variations in water temperature and light intensity. We have measured the stable isotopic composition of specimens of the branching reef coral *Pocillopora damicornis* which have grown in the field while seawater temperatures were continuously recorded. Detailed oxygen isotope profiles of branches of this species indicate that seasonal changes in temperature and seawater isotopic composition are precisely recorded. Isotopic profiles may be used to estimate growth rates of branching corals, which lack annual density banding. The method provides a technique for high resolution palaeoclimatic reconstruction of seasonal temperature ranges and accurate estimation of rates of reef carbonate production.

Thus far, most studies of isotopic and chemical variation in hermatypic corals, and all studies of seasonal isotopic variation, have involved species with massive growth habits, partly because such corals often produce annual²⁴ dense bands which can be used as time markers. For examination of perturbations in climate and ecology of the order of years and decades, large specimens of massive corals which have been growing continuously for as many as 400 yr are available. For high resolution studies on a seasonal scale, however, corals with a branching

growth habit are preferable to massive varieties. Linear growth rates of branching corals are 2–10 times higher than of corals with massive growth habits. We have examined the isotopic response to seasonal changes in temperature, salinity and growth rate of the rapidly growing branching coral, *Pocillopora damicornis*. The advantage of rapid growth is that it permits sampling of many (>20) separate intervals from each year of coral growth, so that short-term temperature changes can be detected.

Our study area was on the south-west side of Contadora Island in the Gulf of Panama. The Gulf of Panama is subject to strong upwelling during the dry season (January to April) when the north-east trade winds shift southward and blow steadily

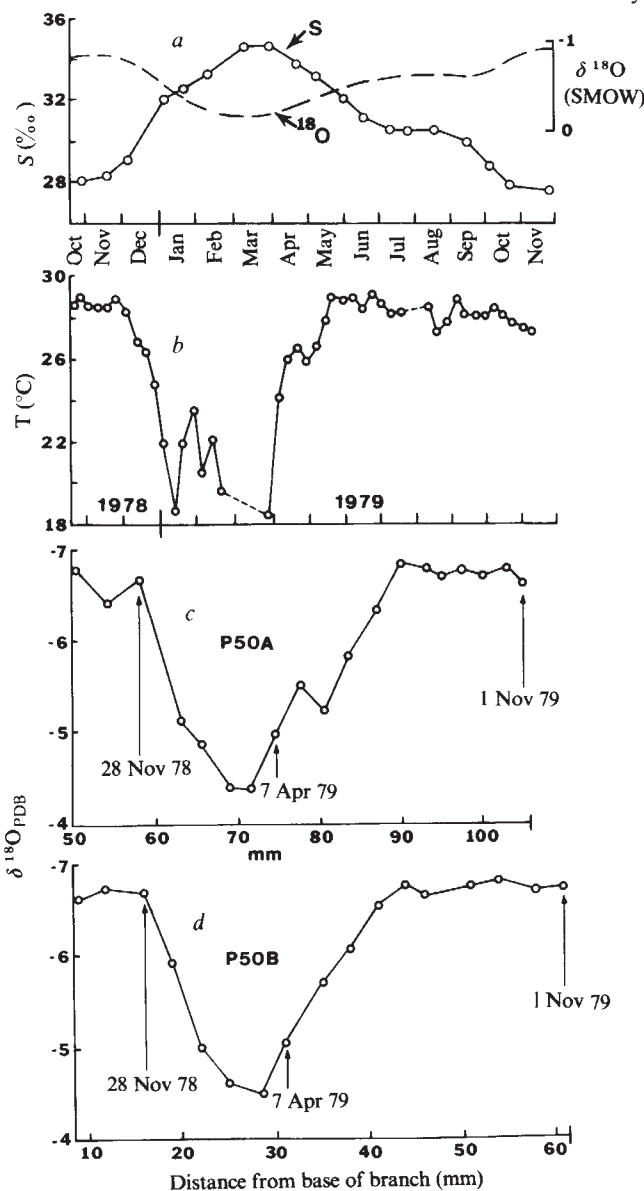


Fig. 1 a, Mean salinity variation from fixed station in the Gulf of Panama near Contadora²⁵ and derived $\delta^{18}\text{O}(\text{‰})$ of seawater based on salinity and $\delta^{18}\text{O}$ measurements of five water samples collected from Naos Island between February and August 1980. For brief periods of time during the rainy season, surface salinity on the reef dropped to as low as 23‰. b, Weekly average temperature recorded on Contadora study reef. We have no temperature record from 19 February to 16 March 1979 due to failure of the thermographs. From 16 March to 6 June and 3 September to 24 November 1979, daily min/max thermometer data are used. Note the positive correlation between low temperature and high $\delta^{18}\text{O}$ of seawater. c, d, $\delta^{18}\text{O}(\text{‰})$ profiles along branches of *Pocillopora damicornis*. Results are relative to the Chicago PDB standard calculated by the usual procedure³². Dates indicate time of staining with dye or harvest.

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across the central Panama isthmus^{7,25}. During upwelling, water temperatures drop to 21–22 °C whereas wet season (May to December) highs reach 28–29 °C. Temperatures in the range 18–20 °C may occur during brief periods when upwelling is very pronounced. Seasonal rainfall begins when upwelling ceases. As a result, salinities average ~27‰ during November and ~34.5‰ during February. Monthly cloud cover ranges from <10% during the upwelling season to >40% at the end of the rainy season⁷.

Branching corals of genus *Pocillopora* are the dominant reef-builders in the Gulf of Panama. Development of the reef framework is generally restricted to the water depths <10 m. Several colonies of *Pocillopora damicornis* on the study reef were protected from cropping by corallivores by completely surrounding them with a wire mesh. The mesh openings were 50 mm, large enough to allow the passage of zooplankton, a potential source of food for the coral polyp (G.M.W. in preparation). These colonies were stained with alizarin-red dye²⁶ on 28 November 1978, and 7 April 1979, to provide time markers for the isotopic profiles and to measure growth rate. Thermographs placed at 3 and 10 m depth on the reef were operated from October 1978 to November 1979, to provide a continuous record of temperature (Fig. 1b). Salinity was periodically measured on the Contadora reef and water samples from Naos Island, Gulf of Panama, were collected to determine the variation in salinity and oxygen isotopic composition of seawater. The stained corals and an additional set of unmonitored corals were harvested from depths ranging from 1 to 4 m on the 1 and 19 November 1979, respectively.

After collection, specimens of *P. damicornis* were soaked in a weak (0.2%) sodium hypochlorite solution and various branches selected for isotopic analysis. The branches were cut in half along their axes of growth. The interiors of the branches were sampled with a dental drill (0.5 mm diameter) every 2–3 mm along recognizable trends of polyp development. Due to the small size of the polyp cavities in these specimens of *P. damicornis*, each sample was comprised of the skeletal elements of several old corallites. Such homogenization of several old corallites reduces the isotopic variability that may result from the inclusion of different skeletal elements (which have different ¹⁸O/¹⁶O ratios¹⁸) in different proportions. About 2 mg of powder resulting from the drilling of each hole was collected and roasted in a vacuum at 250 °C to remove volatile organic compounds before isotopic analysis. The effects of drilling and heat treatment on the mineralogy of the samples were determined by standard X-ray diffraction analysis. Although roasting caused a reproducible (both from branch to branch and in adjacent samples from along an individual branch) conversion of ~10% of the aragonite to calcite, drilling produced no change in mineralogy. Carbonates and seawater were isotopically analysed by standard analytical procedures^{27,28} with a precision of 0.07‰ and 0.05‰, respectively for $\delta^{18}\text{O}$.

The $\delta^{18}\text{O}$ results from two different coral branches grown within the same mesh enclosure are presented in Fig. 1c, d. Both branches show the same seasonal trends, becoming enriched in ¹⁸O during the upwelling season and depleted in ¹⁸O during the wet season. During the height of upwelling, coral growth slows. Previous studies in the Gulf of Panama^{7,8} and Hawaii³ have shown that the growth rate of *P. damicornis* decreases very rapidly between 23 and 21 °C, and is <0.5 mm per month at temperature below 21 °C. Coral growth effectively ceases below 20 °C and 'cold death' will occur if temperatures <18 °C are

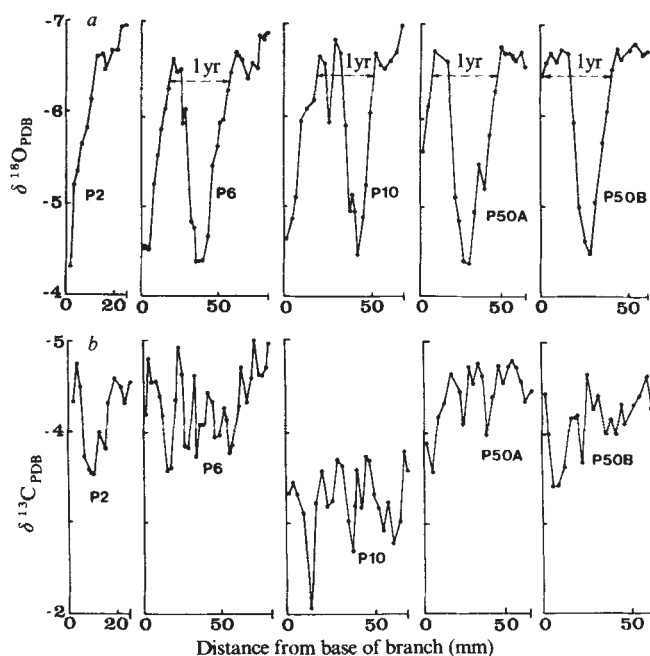


Fig. 2 a, $\delta^{18}\text{O}$ (‰) and b, $\delta^{13}\text{C}$ (‰) profiles in three specimens collected on 19 November 1979 (P2, P6, P10) and branches P50A and P50B collected on 1 November 1979. The start and end of a year of coral growth is arbitrarily set at the levels in branches P6, P10, P50A and P50B, which correspond to coral growth during May (Fig. 1). An annual growth rate for specimen P2 is estimated by correcting the 8-month linear growth rate (based on comparison with the temperature) for seasonal growth rate changes observed in the other specimens. Average growth rates estimated from the $\delta^{18}\text{O}$ profiles are: P2, 29 mm yr⁻¹ (2.4 mm per month); P6, 40 mm yr⁻¹ (3.3 mm per month); P10, 33 mm yr⁻¹ (2.7 mm per month); P50A, 41 mm yr⁻¹ (3.4 mm per month); and P50B, 39 mm yr⁻¹ (3.3 mm per month).

maintained for more than a few days. As a result, the corals retain no isotopic record of periods of intense upwelling. We believe that the isotopically enriched region of these branches represents accretion of skeletal material at 21–22 °C, the average temperature range on the reef between discrete upwelling events during the winter. The maximum linear growth rate, up to 6.5 mm per month (refs 7,8), occurs immediately after the upwelling season, when water temperature rises and near surface productivity is high. Seasonal changes in coral growth rate are illustrated by the compression during February–March and extension during April–May of the isotopic profile relative to the temperature record.

Seasonal rainfall affects the isotopic composition of the coral by changing the isotopic composition of the seawater in which it grows (Fig. 1a). Based on salinity and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ measurements of five water samples taken between February and August at Naos Island this effect, expressed as a function of salinity, is:

$$d\delta/dS (\text{‰}) = 0.12 \quad (1)$$

Although Naos Island is located ~100 km north-west of Contadora, the seasonal pattern of upwelling and rainfall in the two areas is similar. The reservoirs of ¹⁸O in upwelling shelf water and equatorial rainfall are large and should be well-mixed over the short distances between island groups in the Gulf of

Table 1 $\delta^{18}\text{O}$ composition on *P. damicornis*, temperature, salinity and $\delta^{18}\text{O}$ of seawater used to formulate equation (2)

Sample	Temperature (°C)	Salinity (‰)	Seawater $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (‰, SMOW)	Mean $\delta^{18}\text{O}_{\text{coral}}$ (‰, PDB)
28 November 1978 stain horizon P50A, P50B	28.8	28.6	-0.7	-6.68
7 April 1979 stain horizon P50A, P50B	24.0	34.5	-0.2	-5.00
1 November 1979 harvest P50A, P50B	28.2	27.8	-0.8	-6.67
Dry season maxima, P2, 6, 10, 50A, 50B	21.5	33.5	-0.3	-4.40
19 November 1979 harvest P2, 6, 10	28.0	28.0	-0.8	-6.94

Panama. Because of this, the $\delta\delta/dS$ relationship from Naos has been used to estimate average changes in seawater isotopic composition at Contadora.

About 30% (0.7‰) of the seasonal $\delta^{18}\text{O}$ variation in the corals is caused by changing salinity; the remainder is due to a change in seawater temperature of $\sim 7^\circ\text{C}$. Isotopic results on three additional corals (P2, P6, P10) from different areas on the study reef are included in Fig. 2. These specimens were not stained or protected from cropping and were harvested 3 weeks later than branches P50A and P50B. The depletion in ^{18}O near the branch tips of P2, P6, and P10 relative to corals P50A and P50B probably represents a decrease in the ^{18}O of seawater due to increased rainfall during November. Consistent seasonal isotopic variation is present in all five coral branches. The longest record is 22 months (P6). About 21 samples were analysed from each year of coral growth. Using the stain markers and associated $\delta^{18}\text{O}$ values from P50A and B, winter $\delta^{18}\text{O}$ maxima and summer $\delta^{18}\text{O}$ minima from P2, P6 and P10 (Table 1), we calculate an empirical relation of:

$$T(^{\circ}\text{C}) = 7.0 - 3.6[\delta^{18}\text{O}_{\text{damicornis}} - \delta^{18}\text{O}_{\text{H}_2\text{O}}(\text{‰})] \quad (2)$$

Equation (2) fits all data to within 0.5°C . These corals are depleted in ^{18}O by $\sim 3\%$ relative to equilibrium calcite²⁹ (aragonite equilibrium is difficult to determine at low temperatures) and by $\sim 1\%$ relative to results for mixed *Pocillopora* species¹⁶.

Seasonal isotopic variation permits the estimation of net growth rate from $\delta^{18}\text{O}$ profiles (Fig. 2). Although cropping of the unprotected colonies may have occurred, the absence of any marked discontinuities in the isotopic profiles suggests that predation was minimal. While growth rates range from 29 to 41 mm yr^{-1} (2.4 to 3.4 mm per month), the upwelling isotopic maxima and total seasonal range in $\delta^{18}\text{O}$ are equivalent in all five branches depending on the date of harvest. Although these corals are apparently depleted in ^{18}O relative to equilibrium with seawater, we conclude that physiological effects associated with growth rate changes have little effect on the oxygen isotopic response to temperature of this species.

$\delta^{13}\text{C}$ ranges from -3.5 to -5.0% in P2, P6, P50A and P50B and from -2.0 to -3.5% in P10 (Fig. 2b). In general, the corals are depleted in ^{13}C during both the upwelling season and at the end of the rainy season. These are periods of low growth, due to the effects of cold water during upwelling and extensive cloud cover at the end of the rainy season. If the ^{13}C depletion reflects seasonal changes in growth rate, our results are consistent with Goreau's¹⁷ suggestion that rapid growth produces an enrichment of ^{13}C in skeletal aragonite due to preferential removal of ^{12}C by the zooxanthellae. The ^{13}C variations in *P. damicornis* may also be related to a seasonal changes in the $\delta^{13}\text{C}$ of seawater bicarbonate caused by upwelling³⁰ and rainfall.

Fairbanks and Dodge¹⁵ have observed both positive and inverse correlation of ^{13}C and ^{18}O in some Atlantic and Caribbean massive corals. Land *et al.*¹⁸ have observed that rapidly extending parts of individual coral colonies are depleted in both ^{18}O and ^{13}C . $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ are poorly correlated in our specimens. In part this may reflect the occurrence of growth rate minima at times of both cool and warm water. Three branch tips, P2, P6 and P10, are depleted in ^{18}O , probably due to a depletion in the $\delta^{18}\text{O}$ of seawater. No tip is markedly depleted in ^{13}C relative to the rest of the branch. We have no explanation for the enrichment of ^{13}C in specimen P10 although there is no corresponding enrichment in ^{18}O .

We conclude that seasonal temperature changes are accurately recorded by oxygen isotopic variations in branches of the reef coral *Pocillopora damicornis*. Physiological differences between branches apparently have a negligible effect on $\delta^{18}\text{O}$ values, rendering this species useful for the study of modern and ancient seasonal temperature regimes³¹.

Seasonal changes in coral growth rate associated with water temperature and light intensity occur on many reefs. We suggest that such variation must be fully understood before a proper sampling strategy can be formulated for investigations of

seasonal isotopic changes. Branching corals are especially suited to seasonal studies because of their high growth rates which permit sampling of many discrete intervals per year of coral growth. They are important reef builders, especially in thermally marginal regions characterized by seasonal intrusion of cold seawater. In some cases, carbonate production due primarily to branching coral growth exceeds $5 \times 10^3\text{ g CaCO}_3\text{ m}^{-2}\text{ yr}^{-1}$ (ref. 7). Where seasonal temperature variation produces changes in coral isotopic composition which are large compared with salinity effects, $\delta^{18}\text{O}$ profiles may be used to establish time scales in many branching corals, which typically lack annual density banding. Thus the isotopic technique can be used to estimate growth rate and net carbonate production on many modern and fossil coral reefs.

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A fossiliferous borehole section within the Ballantrae ophiolite

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The core recovered during a drilling programme in the Ballantrae ophiolite complex, south-west Scotland^{1,2} includes a 16-m section through highly fossiliferous strata. The borehole containing this section was sunk at North Ballaird (Fig. 1) 100-m west of the abandoned farm buildings [NGR: NX 1210 8777] with an azimuth of 150° and an inclination of 50° . The fossiliferous sequence lies between 24 and 40 m from the surface (125 m above OD) and contains a rich assemblage of Middle Arenig (~ 475 – 480 Myr) graptolites accompanied by inarticulate brachiopods and rare conodonts. Of particular importance is the presence of *Pseudisograptus dumosus* (Harris), an Australasian species not previously recorded from Europe.

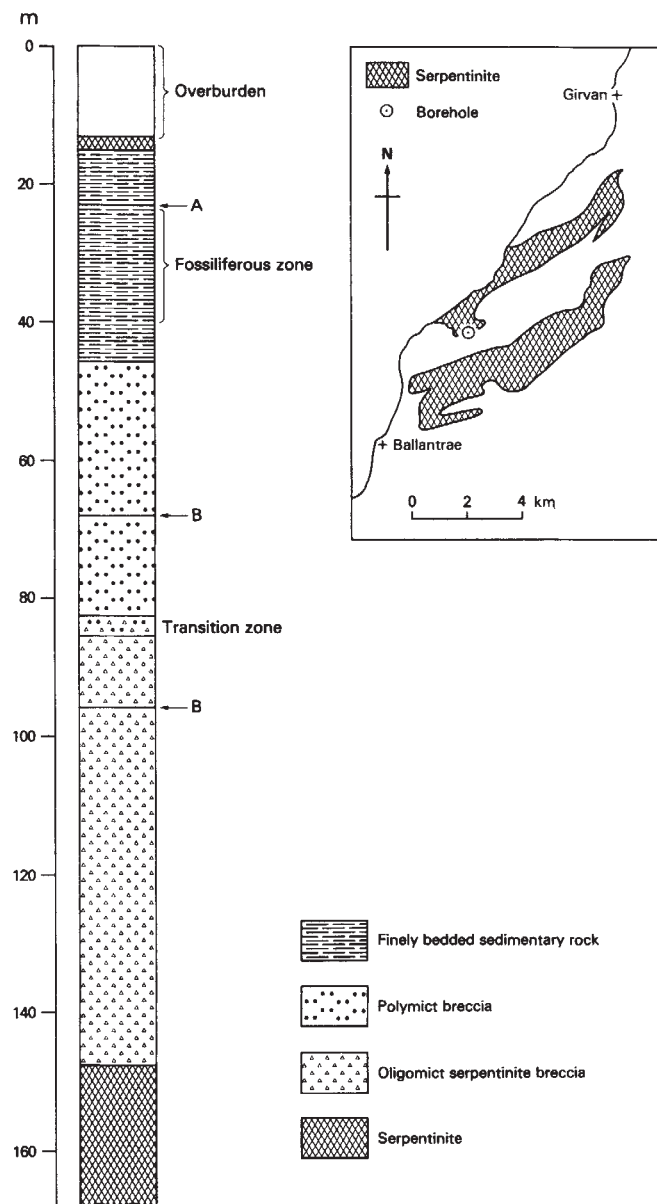


Fig. 1 A summarized graphic borehole log with (inset) a sketch map of the Girvan-Ballantrae area showing the borehole site in relation to the serpentinite belts of the ophiolite complex.

A summarized graphic log of the borehole is shown in Fig. 1. The fossiliferous sediments lie beneath a faulted contact with sheared and carbonate-veined serpentinite. Bedding attitude is approximately normal to the borehole from which a dip of $\sim 40^\circ$ to the north-west can be deduced. The sedimentary sequence consists of a finely laminated succession of chert, shaley chert, siltstone and fine-grained, tuffaceous sandstone. A thin conglomeratic horizon (A on Fig. 1) contains pebbles of a variety of rock fragments, the largest of which are algal-encrusted serpentinite. The bedding in the lowest metre of fine-grained sediments has been disturbed by slumping above a conformable contact with a thick sequence of coarse breccias. In the upper part the breccia unit consists of angular fragments of albitized basalt, dolerite and gabbro ranging up to 8 cm in diameter. These clasts are matrix supported in a fine mudstone. The proportion of matrix decreases downwards and the average size of the angular clasts increases with gabbro becoming the dominant component. At 82.5 m below surface the character of the breccia changes significantly with an abrupt reduction in clast size and angularity and the appearance of serpentinite clasts. The proportion of these increases downwards and below 85.5 m the breccia contains only serpentinite clasts. These have

been extensively silicified and carbonatized, the alteration in places masking the clastic nature of the deposit, but the sedimentary origin of the sequence is emphasized by two thin greywacke horizons (B in Fig. 1). Below ~ 145 m the core consists of unbrecciated but still highly silicified and carbonatized serpentinite.

The graptolite fauna (Fig. 2) is uniformly spread throughout the fossiliferous section of the core and consists largely of isograptids with subordinate didymograptids and other dichograptids. The species include: *Didymograptus* cf. *deflexus* Elles and Wood, *D.* cf. *extensus* (J. Hall), *D.* cf. *protobifidus* Elles, *Isograptus caduceus australis* Cooper, *I.* cf. *imitatus* Harris, *I.* cf. *victoriae lunatus* Harris, *Pseudisograptus dumosus* (Harris), *Tetragraptus pseudobigbyi* Skevington, *T.* cf. *amii* Elles and Wood, *T.* cf. *reclinatus* Elles and Wood, *T.* aff. *serra* (Brongniart), *Phyllograptus angustifolius* J. Hall. There are no diplograptids present. The upper part of the core is richer in species than the lower part and it is possible that more than one zone is represented. It is, however, very difficult to decide what horizon is indicated as the abundance of isograptids and their general Australasian affinities, together with the absence of many critical Australasian forms, makes it impossible to match the associations with published lists. An approximate equivalence with the Middle Arenig zones of *Didymograptus nitidus* and *Isograptus gibberulus* seems to be indicated. The brachiopods are uniformly spread through the fossiliferous section but they are all inarticulate forms and at present are unlikely to be of stratigraphic value. The few conodonts occur above the main graptolitic section. They seem to be fragmentary and even generic identifications are difficult (S. M. Bergstrom, personal communication). The Middle Arenig age, and the Pacific provincial affinities of the graptolites, confirm the conclusions drawn from the sparse fauna previously recorded from the Ballantrae complex^{1,2}.

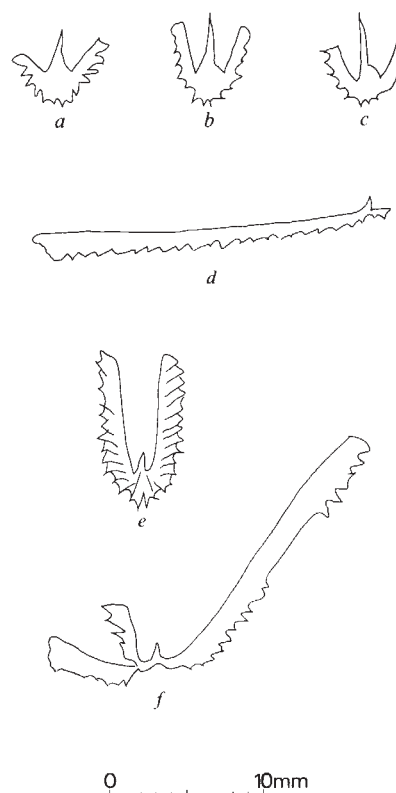


Fig. 2 Graptolite specimens recovered from the core: a-c, *Pseudisograptus dumosus* (Harris). GSE 13724, 13725a, b. d, *Didymograptus* cf. *extensus* (Hall). GSE 13726. e, *Isograptus caduceus* (Salter) s.l. GSE 13727. f, *Tetragraptus* cf. *reclinatus* Elles and Wood. GSE 13728. Numbers refer to specimens housed in the Palaeontology Department, Institute of Geological Sciences, Edinburgh.

The Middle Arenig fossiliferous sequence is clearly of relatively deep-water origin but the interbedded conglomerate contains algal-encrusted serpentinite pebbles which must have been washed in from a shallow water environment. This may have been an oceanic island, a continental margin or a horst within a block-faulted marginal basin from which exposed serpentinite was being actively eroded during the Middle Arenig². The highly angular clasts in the breccias beneath the fossiliferous sequence suggest that basalt, gabbro and serpentinite were exposed on the Middle Arenig sea floor. The upper part of the breccia sequence, in which the clasts are matrix-supported, may well have originated as a debris flow but the lower part of the sequence, where close-packed, large angular clasts of gabbro predominate, is probably a scarp-foot talus accumulation. This would suggest submarine faulting on a fairly large scale which may have developed in association with either a rifted oceanic ridge with a slow spreading rate³ or block faulting associated with the development of a marginal basin. Either situation would be compatible with the deduction of Bluck² that the Ballantrae ophiolite may be compound and was probably structurally complex at the time of its obduction.

The borehole core was made available by Selection Trust Exploration Ltd. The paper appears by permission of the Director, Institute of Geological Sciences (NERC).

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Neogene mass extinction of Western Atlantic molluscs

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There have been many hypotheses for the global mass extinctions that occurred during the Phanerozoic history of the Earth, and although we cannot study the course of such a mass extinction in progress, it is possible to evaluate major pulses of extinction that have taken place recently in geological time. Explanations of the unusually high rates of extinction within late Cenozoic marine faunas of the tropical Western Atlantic have focused on possible blocking of nutrient-laden waters by the origin of the Isthmus of Panama^{1,2} and on Pleistocene cooling^{2,3,5,6} or regression^{2,4,5} of seas. Here we report that major episodes of molluscan extinction occurred at least as far north as Virginia, and we conclude that these episodes were caused primarily by refrigeration associated with the late Pliocene and Pleistocene growth of continental glaciers.

The severity of late Cenozoic extinction in the tropical Western Atlantic has often been underestimated because several young fossil faunas have been judged to be old simply because they include few living species^{7,8}. For example, the enormous molluscan fauna (590 species) of the Bowden Formation of Jamaica was previously considered to be of Oligocene⁹ or Middle Miocene¹⁰ age because ~90% of its species were considered to be extinct. The Bowden's planktonic foraminifera^{11,12}, however, now show it to be no older than zone N19 (early Pliocene), indicating faunal decimation in later Pliocene or Pleistocene time. The incidence of extinction may have been overestimated here because of taxonomic oversplitting, but is certainly high.

The impact of catastrophic extinction on an accurately dated Neogene fauna can be assessed by means of a lyellian percentage: the percentage of the fauna's species that survive to the present day. Lyellian percentages for molluscan faunas of California and Japan form smooth curves (Fig. 1). Because the plots are congruent for the two regions, they appear to represent normal rates of extinction for the two molluscan classes⁸. The composite curve for gastropods lies below that for bivalves, indicating a higher extinction rate.

Figure 1 also displays lyellian percentages for several fossil faunas of eastern North America (Fig. 2). The percentages that represent the Pliocene Epoch fall well below the band of points forming each standard curve, indicating excessive regional extinction late in Cenozoic time. An age of <2 Myr for the Waccamaw and Caloosahatchee faunas^{13,14} indicates that at least some of the extinction took place during the Pleistocene. Lyellian percentages for Miocene bivalve faunas of eastern North America also fall below the standard curve, but this is also a result of post-Miocene extinctions, as shown by the fact that 29% of the bivalve species of the Middle Miocene Calvert Formation of Maryland¹⁵ occur in the Pliocene Yorktown zone 2 fauna of Virginia, which is 10 Myr younger¹⁶. If we treat the latter as a lyellian endpoint, the 29% survival from the middle Miocene to this endpoint is only 8% below the normal lyellian percentage of ~37% for a 10 Myr-old bivalve fauna. The discrepancy can be attributed to imperfection of the Yorktown zone 2 fossil record and to zoogeographic differences between the Calvert and Yorktown faunas. In other words, rates of extinction in the Chesapeake Bay region were little, if any, above normal during the late Miocene.

The extent of the late Cenozoic extinctions is indicated by the presence of ~1,000 bivalve and gastropod species in the

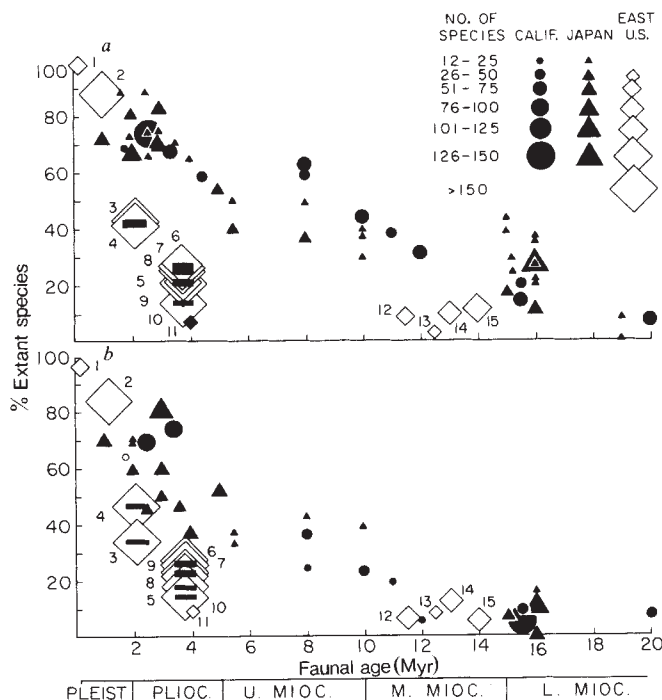


Fig. 1 Lyellian percentages for the Bivalvia (a) and the Gastropoda (b). Points for California (●) and Japan (▲) form tight curves, indicating normal rates of extinction for the two classes⁸. ◇, Faunas of the Atlantic Coastal Plain, from locations shown in Fig. 2; horizontal bars within diamonds indicate the uncertainty of ages. We include no data for the interval between 11 and 5 Myr ago because faunas of this interval are poorly represented in the fossil record and poorly studied. Faunas (by number) and references for age/composition as follows: 1, ref. 49/R. Spencer and L.D.C., in preparation; 2, refs 16/50; 3, refs 51/9; 4, refs 16/16; 5, refs 51/16; 6, refs 52/53, 54, 38; 7, refs 16/16; 8, refs 16/16; 9, refs 16/16; 10, refs 52/16; 11, refs 16/16; 12, refs 55/56, 57; 13, refs 55/56, 57; 14, refs 55/56, 57; 15, refs 55/56, 57.

4 Myr-old upper Pinecrest beds of the Tamiami Formation at Sarasota, Florida, as documented by the Bradley Collection at the Florida State Museum in Gainesville. This approaches the species richness of the entire Caribbean region today¹⁷. Farther north, the entire continental shelf off Virginia today harbours about 220 species¹⁷. More than twice this many fossil species have been collected from adjacent 4-Myr-old Yorktown zone 2 sediments. Even if we assume that every distinction between a Yorktown zone 2 species and a living species that is questionable is invalid, the lyellian percentages for the Yorktown zone 2 faunas remain very low: 20% for bivalves and 15% for gastropods. Clearly the modern Western Atlantic fauna is severely impoverished, and what we have documented, amounts to a regional mass extinction.

We can hypothesize two possible causes for the Neogene mass extinction: first, habitat loss and interspecific crowding due to sea-level lowering; and, second, lethal decline of temperatures. We favour the second hypothesis for the following reasons.

(1) Rate of extinction is normally higher for gastropods than for bivalves (Fig. 1), probably in part because, on the average, gastropod species have narrower geographical ranges⁸. Our set of lyellian percentages for decimated Western Atlantic bivalve faunas, however, is statistically indistinguishable from the set for gastropod faunas. This suggests that the faunas were affected by some agent, like regional temperature change, that was unrelated to normal background causes of extinction. Compression of ranges by sea-level lowering would be expected to have a more severe impact on narrowly distributed species than on broadly distributed species.

(2) Regression alone has failed to cause mass extinction. Pleistocene lowering of sea level in California and Japan produced no excessive regional extinction (Fig. 1), although continental shelves in each area are narrow. Furthermore, at least along the shelf of south-central Japan, it is known that temperatures did not change appreciably across the Plio-Pleistocene boundary¹⁸. Late in Miocene time, there was nearly worldwide regression of seas¹⁹ that affected eastern North America²⁰. The apparent cause of the regression was an expansion of Antarctic glaciation^{20,21} that had virtually no effect on climates of the Northern Hemisphere. As Fig. 1 shows, this regression caused no pulse of extinction in California, Japan or the Chesapeake Bay region.

(3) Nearly all Western Atlantic molluscan species of the last major interglacial stage (Sangamon) are extant²². Examples are species of the Norfolk fauna of Virginia (Figs 1, 2) and the Fort Thompson fauna of Florida. This faunal stability late in Pleistocene time suggests the operation of a threshold effect. In Florida, successive Plio-Pleistocene faunas are separated by unconformities²³. The sequence from Pinecrest to Caloosahatchee to Bermont to Fort Thompson represents the stepwise impoverishment of the enormous early Pliocene Pinecrest fauna (D. Wilson, personal communication). By the late Pleistocene time there apparently remained in the Western Atlantic only species that could tolerate the temperature regimes of glacial stages. Crowding and loss of habitat, in contrast to temperature, are not rigid filters. Had they been the chief sources of extinction, the partially stochastic nature of their operation²⁴ would probably have led to a higher incidence of extinction during the last great glacial regression than the observed 1–3%, which resembles the percentage for stable Pacific faunas (Fig. 1).

(4) A similar extinction event has been documented for Mediterranean bivalves at the time of the Plio-Pleistocene transition. Although not as severe as the Western Atlantic mass extinction, the Mediterranean event was clearly caused by a lowering of temperatures. It eliminated tropical faunal elements and was followed by an invasion of boreal species²⁵.

Continental glaciation began in the Northern Hemisphere ~3 Myr ago and was associated with a pulse of climatic cooling^{26,27}. This is notable because we know that mass extinction commenced along the Atlantic Coast after 4 Myr ago (Fig. 1). Study of planktonic foraminifera indicates that at times during the late Pleistocene, winter sea-surface temperatures in the

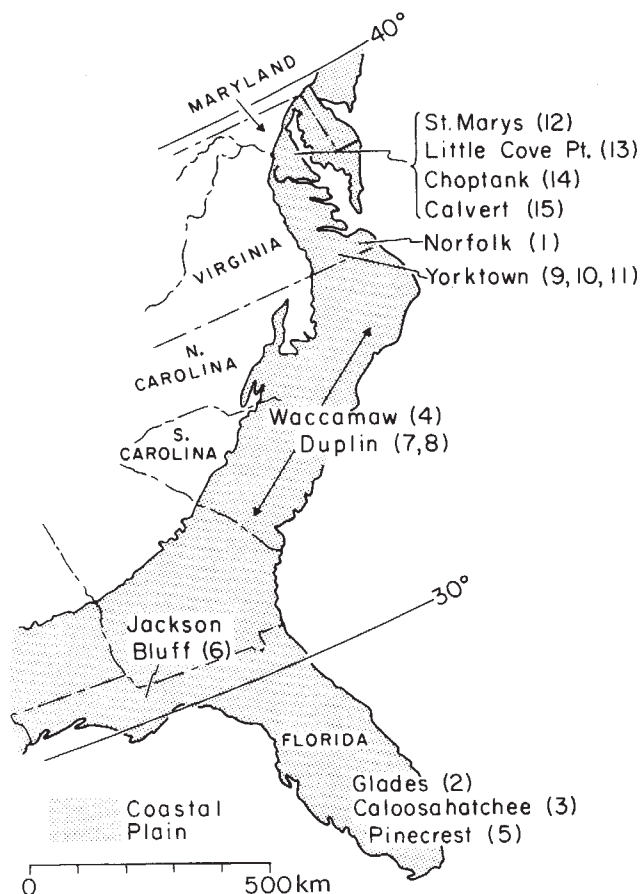


Fig. 2 Geographical location of faunas of the Eastern United States for which data are plotted in Fig. 1.

south-west Caribbean Sea were as much as 4 °C colder than those that obtain there today²⁸, whereas Pliocene temperatures were even warmer than those of the present. Furthermore, regressions during glacial episodes must have accentuated cooling in shallow water, relative to conditions during Pliocene times, by reducing the area of shallow seas, which represent thermal reservoirs. At higher latitudes, in the area south of New England, the results of CLIMAP suggest 'dynamic seasonal change' nearshore during the last glacial interval, with autumns and winters dominated by sea ice and with warmer water moving in during spring and summer²⁹. Such conditions contrast sharply to the subtropical conditions that had been attained in Virginia during latest Yorktown time³⁰. The youngest Yorktown zone 2 faunas have much higher percentages of Caribbean elements than the older Yorktown zone 1 and Miocene faunas of the same region.

The warm interlude represented by the Yorktown zone 2 faunas probably resulted from the closure of the Isthmus of Panama ~3.5 Myr ago. At this time, the Gulf Stream passed far northward along the Atlantic Coast, beyond Newfoundland. Blockage at the Isthmus of Panama is thought to have deflected the North Equatorial Current northwards, strengthening the Gulf Stream³¹. We suggest that this stronger northward flow of warm water gave the Yorktown zone 2 its subtropical character. The warm interlude along the Atlantic Coast was short-lived, however. When continental glaciation began in the Northern Hemisphere ~3 Myr ago, the Labrador Current greatly increased in strength and influence, and this cold, southward flowing current displaced the Gulf Stream to its present, more southerly, position³¹. It was strong southward incursions of the Labrador Current during glacial expansions, and also a general climatic cooling as far south as the Caribbean, that we suggest caused the Neogene mass extinction. Considerably more than 1,000 species of Western Atlantic molluscs were eliminated¹⁶.

Climatic deterioration is one of the least far-fetched mechanisms that can be invoked to explain mass extinctions. Because marine regressions are easily detected in the rock record, they have been cited more frequently than climatic change as possible agents of mass extinction³²⁻³⁹. Nevertheless, while temperature can cause global extinction, regression has never been proved to have this capacity. In fact, when we note that at present the greatest diversity of nearshore benthos is found around oceanic islands, whose sublittoral areas often expand as sea level falls, it is difficult to imagine how worldwide regression can eliminate large taxa simply by causing crowding or loss of habitat on continental shelves. Our data showing great stability for molluscan faunas of California and Japan during the past 3 Myr suggest that regression is not an effective agent of extinction even on narrow continental shelves. Another deficiency of the regression hypothesis is that it fails to apply to either pelagic marine life or terrestrial life, both of which have suffered in many mass extinctions. Climatic deterioration does not share this limitation.

There is evidence that climatic cooling may have been the primary agent of three major pre-Neogene episodes of mass extinction. Significantly, each of these three events struck pelagic as well as benthic marine life: (1) At the end of the Eocene benthic molluscs⁴⁰, planktonic foraminifera^{41,42} and phytoplankton⁴² suffered major extinctions. Evidence from terrestrial floras indicates that a pulse of climatic cooling⁴³ occurred simultaneously. (2) Regressive sequences and tillites now provide good evidence of a glacial episode at the close of Ordovician time, when not only marine benthos but also planktonic graptolites experienced mass extinction⁴⁴⁻⁴⁶. (3) At the end of the Frasnian interval of the late Devonian, both benthos⁴⁷ and the phytoplankton⁴⁸ were decimated on a global scale. At this time, tropical faunas suffered heavy losses (the stromatoporoid-tabulate reef community disappeared altogether), yet the apparently cold-adapted faunas of the Malvinokaffric Province survived near the South Pole⁴⁷ with little effect.

We suggest that climatic change be accorded wider consideration than it has received in the past as a potential agent of mass extinction.

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Are the 3,800-Myr-old Isua objects microfossils, limonite-stained fluid inclusions, or neither?

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Bridgwater *et al.*¹ issued a 'cautionary note' concerning several reports published by Pflug and co-workers²⁻⁵ describing objects called yeast-like microfossils (*Isuasphaera isua* Pflug) from a metamorphosed quartzite of the 3,800-Myr-old Isua supracrustal belt of south-west Greenland; Bridgwater *et al.* believe that the objects described by Pflug *et al.*²⁻⁵ are 'indistinguishable from limonite-stained fluid inclusions' and hence are non-biogenic. I show here that the objects are neither limonite-stained fluid inclusions nor microfossils, but are limonite-stained cavities from the otherwise complete dissolution by weathering of ferruginous dolomite grains in these rocks. Several supporting arguments presented by both sides are believed to be invalid, and others are ambiguous. In view of the extensive research on the earliest life forms, and their significance to evolution, to early geochemical cycles and to the origin of the atmosphere and some ore deposits, the exact nature of the Isua objects, and particularly the validity of the evidence either for or against a biological origin, are of considerable importance. A careful evaluation of the evidence from Isua is particularly pertinent, as *bona fide* Precambrian fossils are also found in chemically similar (but much younger) silica-rich environments.

I have examined the following sections of the original rock in question (no. 2377): 2377-1-D from J. W. Schopf, UCLA; and 2377, 2377-G₁ and 2377-G₂, and a small piece of the rock itself, from H. D. Pflug. The objects vary only in abundance among the four sections.

A brief description of the 'fossil-like' objects is necessary, because some of those described by Bridgwater *et al.*¹ are not of the same type as are those described by Pflug *et al.*²⁻⁵. The latter are irregularly ovoid features, ~10-40 µm in length, which are distinguishable from the embedding quartz on the basis of two features: (1) the walls of the ovoids are coated and hence outlined by a granular substance having a relatively high index of refraction (*n* much greater than that of quartz); some ovoids are partly filled by this substance; and (2) this substance is dark yellowish brown.

Bridgwater *et al.*¹, however, described two quite different 'categories' of 'fossil-like' objects. One is yellowish to reddish brown (here called brown objects), and corresponds exactly to the objects described by Pflug. The other are "... clear, spherical to ellipsoidal structures (Fig. 1b), occur only within quartz grains (rather than at grain boundaries); these structures are comparable to fluid inclusions ..." (here called clear objects). They are always closely associated with the brown objects (Fig.

1b, c), but in all the many photomicrographs of *Isuasphaera isua* presented by Pflug *et al.*²⁻⁵, not a single clear object is seen. Although they did not so state explicitly, the context indicates that Bridgwater *et al.*¹ believed that the brown objects are the exact equivalents of the clear objects, but as a consequence of occurrence on grain boundaries, the brown objects have become iron stained. I hope to show first that this equivalence is indeed correct, but then to show that neither brown nor clear objects are, or were, fluid inclusions or microfossils.

Not being a palaeontologist, I am not qualified to discuss the various morphological features within the objects that are described by Pflug *et al.*²⁻⁵ as biogenic structures, except to note that at least some of the Isua objects each appear to my untrained eye to be extremely similar to at least one or more of a series of previously described (and more generally accepted) Precambrian microfossils⁶⁻¹⁰. As a result of my petrographic examination, however, I present the following observations that are pertinent to the origin of the Isua objects and may also have some applicability to samples from other localities.

(1) Deformation of the host material: the bulk of the evidence presented by Bridgwater *et al.*¹ to negate a biological origin for the brown objects is based on the premise that such fossils could not be preserved in rocks that had undergone such an extensive series of high-grade metamorphic and tectonic events.

The amount of deformation in any compositionally banded metamorphic rock will vary widely with the nature of the assemblage; quartzites are among the most resistant bands and frequently form boudins, and hence should be the most resistant to the destruction of any fossils (macro or micro). The quartz in sample 2377 does show some preferred orientation, from an unknown stage in the metamorphic history. This was best seen by a simple optical integration procedure: view in crossed polars with the 1λ plate, at very low magnification and grossly defocused. During rotation of the stage, an overall change from bluish to yellowish is readily visible.

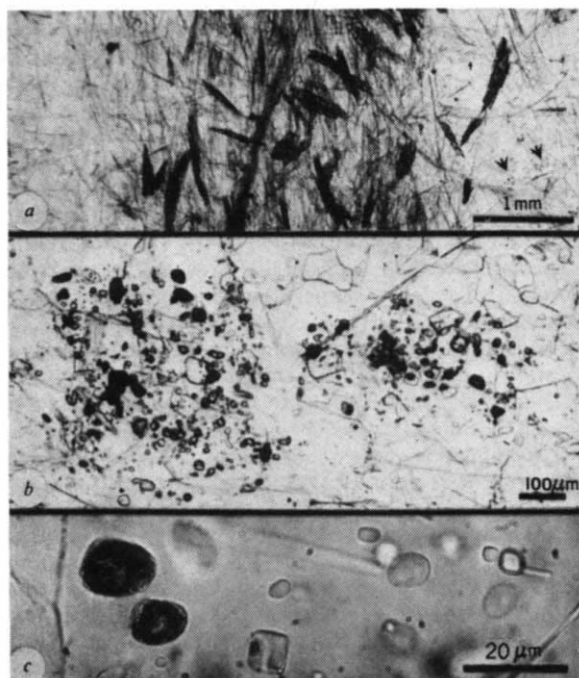


Fig. 1 *a*, Photomicrograph at low magnification of actinolite-rich band between two quartz-rich bands in sample 2377. Two small clusters of brown objects are visible (arrows). *b*, Two clusters, containing respectively 46 and 27 brown objects (photographed in green light, so they appear opaque), along with many clear objects (here mostly silicate crystals) in an area of quartzite sample 2377 that is free of all but a few small actinolite needles. Note also the lack of 'limonite stained grain boundaries'. *c*, Enlarged view of a part of a cluster similar to those shown in 1*b*, showing two brown objects and many clear objects.

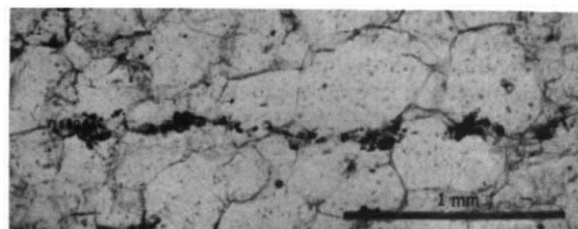


Fig. 2 Band of brown and clear objects that appears to cut across quartz-grain boundaries in actinolite-free quartz in sample 2377-1-D.

(2) Distribution of the objects: most of the clear and brown objects occur in a very small fraction of each section, in scattered clusters or bands of larger quartz grains (~100–500 μm in diameter). These particular bands have little or no actinolite (Figs 1*a*, 2).

(3) Nature of the clear objects: I find most of the clear objects to be crystals of one or more silicate minerals and not fluid inclusions (Fig. 1*c*). They are ovoid single crystals (some are slightly greenish) having an index of refraction *n* much greater than quartz and moderate to high birefringence, and in polished sections they are more reflective than the host quartz. The clear objects that are not crystals of silicate minerals are ovoid, single crystals of a rhombohedral carbonate mineral, presumably ferrous dolomite. Some of these, in turn, have one or more small (~1–2 μm) fluid(?) inclusions attached to the interface with the host quartz; these inclusions may be high-pressure gas, as the quartz enclosing some of the carbonate inclusions shows strain birefringence, and many of the carbonate inclusions have decrepitated to form pits where they are cut by the polished surface. Identification as dolomite was based on optic sign, on estimates of the birefringence and the indices of refraction relative to those of quartz, and on solution in HCl under the microscope, accompanied by very slow effervescence, of three small grains that were at the surface. The presence of dolomite in contact with quartz places limits on the maximum pressure-temperature combination of the metamorphism. These two minerals react to form diopside plus CO₂ at 2,000 bars at ~520 °C (ref. 11); each 20 °C above 520 °C increases the pressure another 1,000 bar. I do not know why these few grains have not reacted, whereas other presumed grains have reacted to form actinolite.

(4) Nature of actual fluid inclusions present: only a relatively few isolated true fluid inclusions were found. These are at most only a few micrometres in length (Fig. 3); they are irregular or faceted but not ovoid; they consist of two isotropic phases (liquid and a gas bubble, which is frequently in motion); *n* is much less than quartz; and at a polished surface, they would be represented only by holes. None was found in the size range (and shape) of the clear or brown objects. Hence I suggest that the two clear objects illustrated by Bridgwater *et al.*¹ in their Fig. 1*b*, which they called fluid inclusions, may be silicate or carbonate crystals rather than fluid.

(5) Distribution of objects relative to quartz grain boundaries: the specific nature of the occurrence of the objects—on grain boundaries or within single quartz crystals—is critical to the interpretation of many of the data obtained, and is surprisingly difficult to determine unambiguously. Pflug *et al.*⁵ stated that some of his brown objects were actually within single crystals of quartz, whereas Bridgwater *et al.*¹ stated that the brown objects "... show a marked concentration at quartz grain boundaries ...". The importance of this distinction lies in the fact that if a brown object were actually enclosed within a single crystal of quartz, both deformation and chemical metasomatism of the surrounding rocks would be of no consequence to its preservation unless that quartz crystal were deformed and recrystallized. A close analogy is the preservation of the spiral growth patterns in some metamorphic garnets¹². However, the

apparent occurrence of some brown objects *within* quartz crystals is only apparent, as shown by the next observation.

(6) Solubility of the brown material: I gently crushed ~100 mg of sample 2377 to <~1 mm, immersed it in 1.55 index oil, and picked out ~50 grains, each containing one or more typical brown objects. After removal of the oil, I covered these grains with dilute HCl (20 vol.% concentrated HCl, 80 vol.% H₂O) at 22 °C. All the brown objects (~250) and brown grain boundaries (Fig. 4) lost their brown colour completely within 30 min, leaving cavities partly or completely filled by clear or slightly yellowish acid. Organic materials in sediments, particularly old sediments, are generally considered to be relatively immune to attack by HCl, and this insolubility, even in hot, concentrated HCl (±HF) is commonly used as the basis for a laboratory technique for concentration of the organic matter¹³. Because the brown objects were readily soluble in cold dilute HCl, and because a yellow solution remained in the cavities, I conclude that the brown material in the objects I have examined is not organic and is probably limonite. Even more important to other studies of possible Precambrian organic matter is the fact that these brown objects were not completely enclosed within single quartz crystals, even though they appeared to be. From the fact that clear objects, of both carbonate and silicate types, were not affected by the acid, I conclude that every dolomite grain that was not completely enclosed in an unfractured single quartz crystal has been weathered to yield a limonite-stained cavity, that is, a brown object, and I conclude that the same, in part optically invisible pathways permitting early entry of the weathering solutions also gave access to the HCl.

The above conclusion concerning the nature of the Isua brown objects requires some disposition of several other lines of evidence proposed to support each of the other two theories:

Bridgwater *et al.*¹ interpret the 'budding' reported by Pflug *et al.*⁵ as a 'necking-off' process commonly occurring in inclusions, and refer to my own work. Unfortunately, however, the most diagnostic feature that is routinely used by students of fluid inclusions to recognize arrested necking-off—the common occurrence of pairs of individually isolated, generally ovoid inclusions, each having a sharp projection pointing towards the other of the pair¹⁴—was not seen in any Isua brown (or clear) object. I interpret the budding as the result of the weathering of touching composite grains, as many almost identical shapes can be found among unweathered crystal inclusions.

Bridgwater *et al.*¹ state that the reported 'gas vacuoles' (see their Fig. 1f) are bubbles trapped within the objects during their formation and recrystallization, that is, primary gas inclusions, but I find no evidence of trapping of primary gas. The high metamorphic pressures they suggested also tend to preclude trapping primary gas, and no such bubbles are found in the clear objects.

They also found brown objects with rhombohedral, obviously crystalline shapes (see their Fig. 1e). These may well represent former carbonate crystals. The exact mechanisms whereby soft microfossils become preserved in crystalline silica are obscure¹⁵, but there seems to be no doubt that such structures are pre-

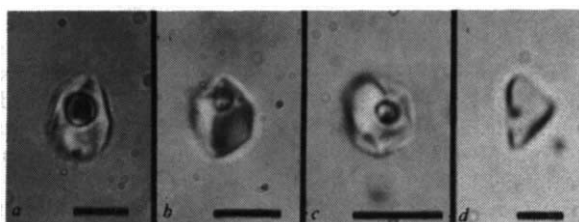


Fig. 3 a–d, Four typical aqueous fluid inclusions from sample 2377-1-D. The smaller vapour bubbles are in constant motion (particularly that shown in d) and hence are not in sharp focus. These inclusions would homogenize over a wide range of temperature; they all are probably secondary. Scale bar, 5 μm.

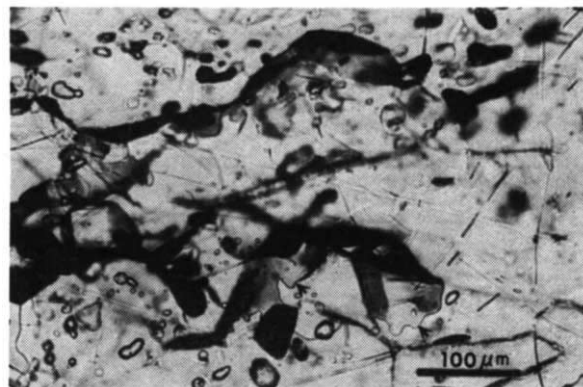


Fig. 4 Cluster of brown and clear objects in sample 2377 in which some dark olive-brown matter apparently has leaked out along the grain boundaries (arrows).

served in younger rocks, though the reading of the record is fraught with difficulties¹⁶. However, I see no difficulty in the presumption that the cavity representing a microfossil within a quartz crystal could become faceted and hence could represent merely an intermediate stage in its destruction. Similarly, I see no difficulty in the presumption that actinolite needles could have grown in such a way that they appear to penetrate the brown objects, regardless of their origin.

Pflug *et al.*⁵ stated that the brown material is more resistant than quartz to attack by hot vapours of HCl and HF; this is to be expected for limonite, as iron chlorides and fluorides are not volatile as SiF₄ is.

Pflug *et al.*⁵ reported that brown objects (supposedly *within* single quartz grains) showed weak fluorescence, and extensive optical, IR absorption and Raman spectral studies by Pflug *et al.*^{2–5} showed a series of organic components in the brown objects. I suggest that these data may stem from natural organic contamination during weathering, or even from migration of the indigenous, 3,800-Myr-old organic matter reported to exist in these rocks^{17,18}. The extremely weak spectra expected from major limonite could easily be lost in the very strong signals expected from trace organics.

The 'multilaminant sheaths' (that is, multiple layers of brown material on the walls) reported and illustrated by Pflug *et al.*^{2–5} may represent several stages of deposition of limonite.

The several brown objects that also contain liquid and vapour in the central cavity (ref. 1, Fig. 1f; ref. 5, Plate 2, Figs 4 and 8) may well be cavities formed by weathering of dolomite grains that have since been partly resealed by precipitation of silica (the highly variable gas–liquid ratio ratio suggests that this sealing is poor).

Many Phanerozoic fossils have been infilled with calcite or other minerals, and subsequently leached, but these occur in permeable, polycrystalline rocks, not within single crystals.

Even though I conclude that the Isua objects are not microfossils, I am confident that organic material, and even morphologically recognizable fossils, if actually trapped *within* single crystals, should be able to withstand rather high grade metamorphism. The ability of organic matter to persist is evident from the fact that coalification of organic matter does not proceed as far in a given set of conditions if the gaseous products cannot escape as it does if gaseous products can escape¹⁹. I also conclude that incipient weathering (and in these samples, even complete dissolution of dolomite grains) can take place through seemingly tight grain boundaries and even through invisible fractures in apparently 'unweathered' rocks. The most important conclusion, however, is that considerable caution is called for in the study of Precambrian microfossils.

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The earliest seeds

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Lagenostomalean-type seeds in bifurcating cupule systems have been discovered in the late Devonian Hampshire Formation of Randolph County, West Virginia, USA (Fig. 1). The associated megafloora, plants from coal balls, and vertebrate and invertebrate faunas demonstrate that the material is Famennian; the microflora indicates a more specific Fa2c age. Consequently, these seeds predate *Archaeosperma arnoldii*¹ from the Fa2d of northeastern Pennsylvania, the oldest previously reported seed. By applying precision fracture, transfer, dégagement, and thin-section techniques to selected cupules from the more than 100 specimens on hand, we have determined the three-dimensional morphology and histology of the seeds (Fig. 2a–h, k) and cupule systems. A comparison with known late Devonian to early Carboniferous seeds reveals that ours are more primitively organized than all except *Genomosperma*^{2,3}.

Cupules terminate a bifurcating branch system. In the more extensive specimens the bottom forks are relatively equal while more distal forks are less so and occur at progressively closer intervals. The cupules (Figs 2a–c, 3), ~1-cm long and apically flared to ~1-cm wide, also consist of a bifurcating system but are highly condensed and easily distinguished from the rest of the branch system. The cupule base bifurcates twice at right angles to form four quarters (Fig. 3). Each quarter bears a seed towards the centre of the cupule and has four sterile telomes towards the exterior (Figs 2a, b, 3). Thus a complete cupule has 16 free apical tips surrounding the seeds. All parts of the cupule, including the seed stalks, are supplied by small, terete, centrarch xylem strands of annular to scalariform tracheids. Compressed cupules closely resemble those of *Archaeosperma*¹, but when preserved in a relatively uncompressed state (Fig. 2k) the free tips surround a single seed-containing cavity. Our cupule branch systems also resemble *Moresnetia*⁴ and individual cupules are similar to those of *Eurystoma* and *Stamnostoma*^{2,3} and to *Hydrasperma*^{5,6} but are more obviously dichotomous than these. This reinforces the interpretation that lyginopterid cupules originated from systems of dichotomous branchlets.

The radiospermic seeds are 5–6 mm long and taper from ~2 mm in diameter in the midregion to ~1 mm at the base (Figs

2b, c, 3). The 4–5 parted integument is smooth and fused to the megasporangium in the basal 1/3 of the seed (Figs 2c–g, 3). Distally the integument divides into separate lobes that gradually curve inwards at their tips (Figs 2b, c, 3). The individual lobes are free from one another slightly below the level where each separates from the megasporangium. Externally the degree of integument fusion (connation) is only slightly greater than that of *Genomosperma latens*^{2,3} while internally the degree of fusion to the nucellus (adnation) is more like that of *Salpingostoma*^{2,3} or *Tantalosperma*⁷. The top of the nucellus (megasporangium) bulges conspicuously into the space encircled by the free tips of the integument lobes (Figs 2b, d, 3), and its apex is elongated into an apparent salpinx-like tube (Fig. 2d). Below the pollen chamber (Fig. 2h) the nucellus consists of several layers of delicate cells covered by an epidermis of radially elongated cells and surrounds a prominent megaspore membrane.

The seed-bearing cupules are most abundant in the underclays and roof shales of two 10-cm thick coal beds separated by 15 m of sediment. The underclays contain an upper layer with many rooted axes of *Rhacophyton* and grade downward into siltier layers in which, besides *Rhacophyton*, are found numerous cupules, pinna and pinnules of *Sphenopteris*, a lycopod, fish scales, and linguloid brachiopods.

The coals, formed primarily from autochthonous *Rhacophyton* fragments, contain partings of fine clastics and are highly pyritic. The pyrite has sometimes petrified individual axes of *Rhacophyton*, but in other cases has enclosed masses of uncompressed *Rhacophyton* (Fig. 2i, j) in flattened concretions which represent the oldest coal balls ever found⁸. These coal balls are further unusual in that nearly all consist of a lower layer of uncompressed autochthonous *Rhacophyton* peat and an upper layer of allochthonous marine clastics containing compressed plant detritus, fish scales, and occasional brachiopods (Fig. 2i, j). Coal balls with a similar mixture from Pennsylvanian-age coals have been called 'heterogeneous mixed'⁸.

The associated flora of the interbedded deltaic deposits consists mostly of the progymnosperm *Archaeopteris* (*A. macilenta*, *A. halliana*, *A. obtusa*, *A. hibernica*, and *A. sphenophyllifolia* and *Callixylon erianum*) and the zygopterid fern *Rhacophyton ceratangium*. These assemblages occur in distinct layers and pockets. Other less common plants include branch systems with synangia like *Aneurophyton olnense*; petrified *Hierogramma*; a lycopod with vertically elongated leaf scars like *Helenia*, *Heleniella*, or *Protopinakodendron*; *Barinophyton*; *Xenotheca*; *Condrusia*; *Sphenopteris*; and the cupules. The plants and animals are distributed in this sedimentary sequence in characteristic patterns. Continued study should help us to understand the palaeoecology of these earliest seed plants.

The Hampshire Formation in this part of West Virginia (Cassadaga Stage, Chautauquan) is generally regarded as mid to late Famennian in age⁹. The megafloora fits Banks¹⁰ assemblage VII (mid Famennian to basal Tournaisian), but the flora is

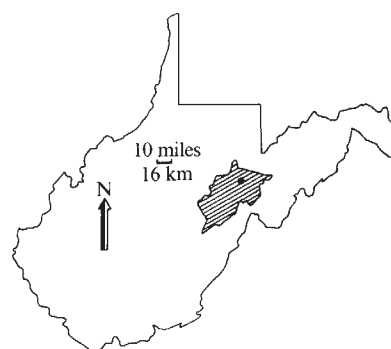
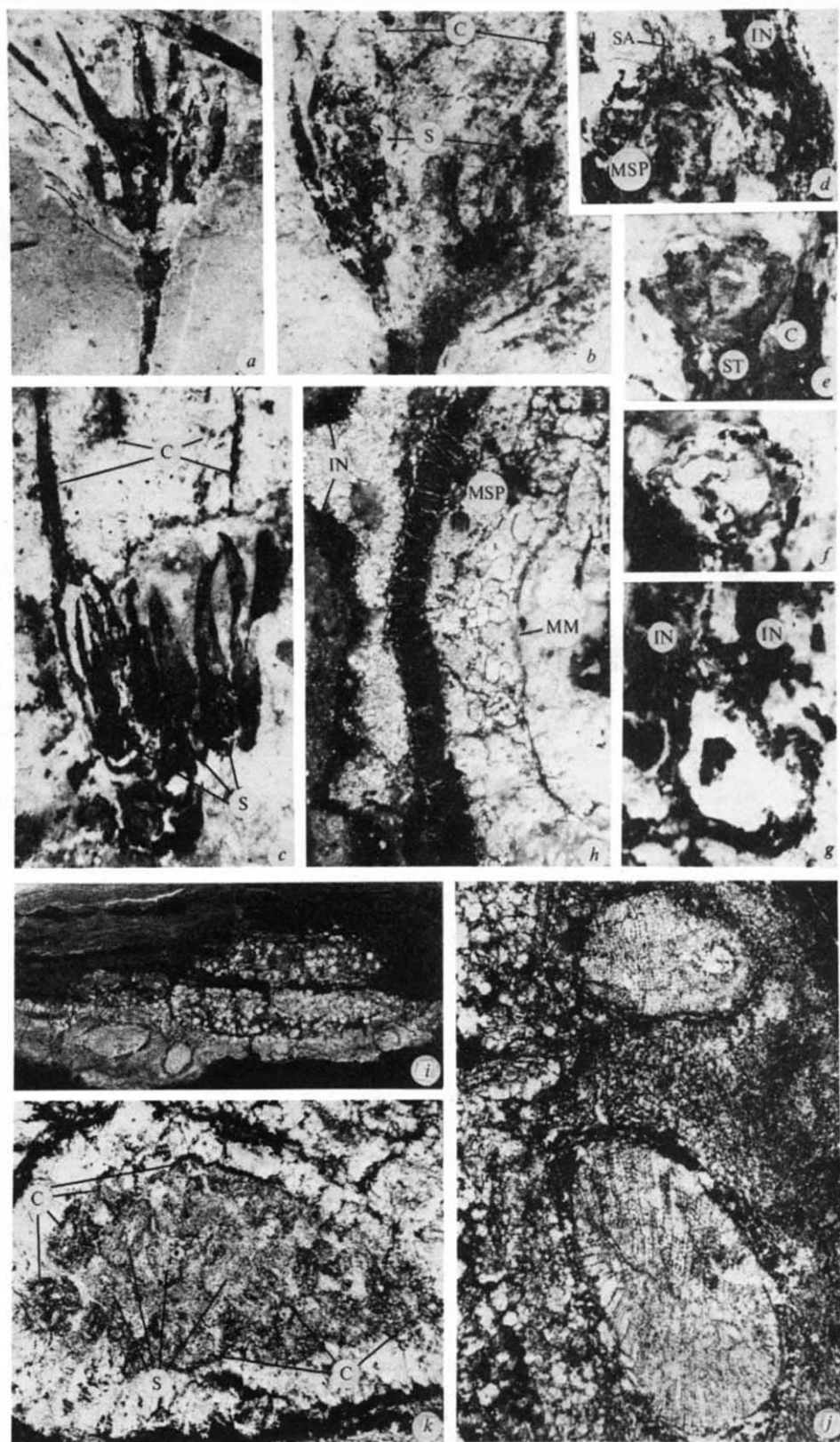


Fig. 1 State of West Virginia with Randolph County lined and locality dotted.

Fig. 2 *a*, Exterior of a cupule showing parts of two quarters. $\times 3$. *b*, Interior of the same cupule (C) showing the attachment of two seeds (S), one to each quarter. Left seed, seen in median longitudinal fracture, shows the shape of the megasporangium and its bulging apex. $\times 6$. *c*, Interior of another cupule (C) with three nearly complete seeds (S) showing the free integument lobes. $\times 6$. *d*, Counterpart of the megasporangial apex (MSP) of the left seed in *b* to show the bulged dome terminated by a salpinx-like tube (SA) and the inner surface of one integument lobe (IN) that curves over it. $\times 20$. *e*, Cross-section fracture of the base of the middle seed (partially petrified) from *c* to show its circular outline at this level and its stalk (ST). $\times 21$. *f*, Cross-section fracture of the same seed, but higher, to show its four-angled integument and megaspore. $\times 21$. *g*, Oblique cross-section fracture below the middle of the left seed (partially petrified) in *c* to show the four-ridged integument separating into free lobes above this level (two IN). $\times 21$. *h*, Cross-section near the middle of a petrified seed to show integument lobes (IN), part of the free megasporangium (MSP), and some of the megaspore membrane (MM). $\times 95$. *i*, Etched coal ball slice to show coal (bottom), uncompressed autochthonous *Rhacophyton* peat (middle), and compressed allochthonous marine detritus (top). $\times 3$. *j*, Detail of the *Rhacophyton* axes from *i*. $\times 13$. *k*, Cross-section of a petrified cupule (C) from a coal ball to show exterior cupule lobes surrounding several seeds (S) cut near their bases. $\times 13$.



almost identical to that from the Assise d'Evieux of Belgium⁴ which is mid late Famennian (Fa2c). The fauna is biostratigraphically less useful but supports a late Devonian designation. Another nearby section of the Upper Hampshire^{11,12}, but with only *Rhacophyton* and *Archaeopteris* present, has yielded a microflora age of lower Fa2c (VU_i in European spore zones)¹³, but is probably slightly younger than our locality (J. F. Schweitering and J. M. Dennison, personal communications). A

preliminary palynological analysis suggests that our material is no younger than upper Fa2c (VUs) (G. D. Wood, personal communication).

If this biostratigraphic analysis is correct, then our seeds are significantly older than *Archaeosperma arnoldii* from the Oswayo Formation of Pennsylvania¹. The Oswayo microflora from adjacent stratigraphic sections in New York suggests an age no older than Fa2d (PL_i) and is probably at the top of the

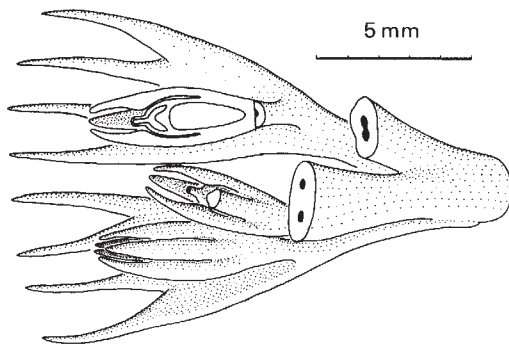


Fig. 3 Reconstruction of parts of a cupule with three seeds; xylem in black.

Fa2d (G. D. Wood, personal communication). According to palynological studies¹⁴⁻¹⁶ other seeds reported from late Devonian rocks of southern Ireland (*Spermolithus devonicus*¹⁷ and *Hydrasperma tenuis*⁵) are probably even younger.

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Microwear polishes on early stone tools from Koobi Fora, Kenya

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The functions of the stone artefacts made and used by early hominids has been a matter for speculation. However, recent experimental work has demonstrated that microscopically distinct wear-polishes form on tools of cryptocrystalline silica when used on different materials, and that these microwear polishes survive on ancient implements¹⁻³. We have now examined 54 artefacts from five early Pleistocene archaeological sites, dated to 1.5 Myr ago, in the Koobi Fora region of Kenya for microwear polishes and other traces of use. Wear traces were found on nine artefacts, variously resembling traces induced experimentally by cutting soft animal tissue and soft plant material and by scraping and sawing wood. These results greatly extend the time range for which microwear polish analysis is applicable and increase the evidence of early hominid adaptation.

Four of the sites sampled were stratified in fine-grained floodplain deposits adjacent to ancient stream courses (FxJj 18IH, 20M, 20E and 50) and one (FxJj 18GS) was found in river gravels⁴. At sites 20 and especially 50, artefacts have been fitted together to reconstruct total and partial knapping episodes⁵, demonstrating that they were little disturbed by natural forces during or after burial. This conclusion is supported by all other pertinent archaeological and geological evidence. Site 18GS is an exception and, while most artefacts from this location were naturally abraded, one unabraded chert artefact (discussed below) was anomalously fresh.

The assemblages from all these sites are attributed to the Karari Industry⁶, which is regarded as a regional variant of the Olduvian Industrial Complex⁷. K-Ar radioisotope analysis of volcanic marker tuffs, stratified above and below these sites, date them to ~1.5 Myr ago⁸⁻¹⁰.

More than 90% of the artefacts at each site were made of basalt. The surfaces of the basalt pieces had undergone a light chemical dissolution ('weathering'), making them very unsuitable for microwear analysis. A few more resistant materials, including cryptocrystalline silicas and ignimbrites, are found at these and most Koobi Fora sites. Fifty-four non-basalt artefacts, each with one dimension larger than 2 cm and at least one non-cortical edge, were selected (by N.T.) from the five Karari assemblages for microscopic study. Except for one minimally flaked pebble core, all were flakes and flake fragments. A few were retouched.

When examined microscopically, none showed any traces of natural abrasion by wind or water and only five bore evidence of slight chemical weathering (none of these is discussed below). The ridges and edges of all but the latter were microscopically sharp and, at ×400 magnification, were indistinguishable from the edges and ridges of freshly struck modern flakes of similar materials. This finding implies that the original surfaces of these artefacts and any wear traces they might bear are likely to have survived intact.

As previous microwear analysis has involved tools of European flint used on materials from a temperate environment¹⁻³, we 're-calibrated' our inferential base by using experimental tools of Koobi Fora and European cryptocrystalline rocks on plant and animal materials (including the carcass of a circus elephant) from the tropical savanna. (The experiments were conducted by N.T., except for the elephant butchery, at Koobi Fora on local materials.) Wear traces found on the Koobi Fora experimental tools were identical to those seen on European implements used in analogous ways with two exceptions: (1) the flake surfaces of some of the Koobi Fora chert tools (both experimental and archaeological) show a natural 'greasy' lustre resembling the microwear polish created by low-intensity meat-cutting, effectively raising the threshold of detection for the latter, and (2) the modern grasses cut at Koobi Fora induced the formation of a 'soft-plant' polish much faster than temperate grasses (10 min of use at Koobi Fora being the equivalent of >1 h of use in Europe).

Both experimental and archaeological specimens were cleaned with ammonia-based detergent, H₂O₂ and dilute HCl. They were examined with an incident-light microscope at magnifications of ×50-×400 before, during and after cleaning. Functional interpretations were based on the reflectivity and texture of the microwear polish, the size and direction of micro-scratches ('striae'), the size and character of the edge damage, and the distribution of all these traces along an edge^{1,2}. Nine implements in the sample showed clear microwear traces.

Four implements, three from site 50, showed a rough-textured, 'greasy' microwear polish along one of their edges (Fig. 1c, d) similar to that induced experimentally by cutting meat and other soft animal tissues. Striae running parallel to the edge and a symmetrical distribution of polish on both edge aspects indicate that all were used with a slicing motion. Coincident with the areas of polish, all the edges bore microscopic damage scars that are typical features of edges used for cutting soft material. Three of the tools have cortical surfaces opposite the used edge

that may have served as handles during use. After interpreting the site 50 tools as meat-cutting knives, it was found that two of them were recovered within 1 m of a large bovid humerus showing narrow cutmarks, presumably from a stone knife (ref. 5 and E. Kroll, personal communication).

Two implements had, along portions of their edges, a highly reflective, smooth-textured microwear polish of the type produced by use on soft plant material containing substantial amounts of plant silica (Fig. 1a, e). The intensity of the polishes,

in contrast to the minimal edge-damage indicating brief usages, suggest that these implements were used to cut the highly siliceous stems of grasses or reeds. Examination of the polish surfaces at higher magnifications with a scanning electron microscope, following the method of Anderson¹¹, may reveal undissolved, identifiable phytoliths that could narrow the possibilities. Both pieces bear evidence of use with a slicing motion—striae parallel to their edges and wear traces symmetrically distributed on both edge aspects. The used edges also show the minute damage scars typically resulting from the cutting of soft material. These two tools provide the first direct evidence that early hominids used stone tools to gather or process plant material.

Three pieces show wear polishes of the type produced experimentally by working wood, which resemble those found on implements used for working soft plant material but are less reflective and less developed (Fig. 1b). Two of these tools were used as scrapers, as they have polish rounding the edge from the ventral aspect, striae at high angles to the edge and 'stepped' damage scars on the dorsal edge aspect. The other implement shows symmetrical wear traces, parallel striae and a few crescent-shaped damage scars, indicating its use as a saw. Except for 'hammerstones' (naturally shaped pebbles used to strike off flakes), these three implements may represent the earliest identified 'tools to make tools'.

Although preliminary, our results are pertinent to the controversy concerning the diet of early hominids and the role of stone tools in their adaption. The detection of wear traces indistinguishable from experimental meat-cutting polishes is strong evidence that early hominids ate meat. Our use-wear data independently corroborate the evidence of the cut-marks found on bones from several early sites, including some of those we sampled. Our evidence documents the use, by early hominids, of stone tools to cut soft plant material, but does not indicate the purpose of this activity. Our identification of a few wood-working tools indicates that by 1.5 Myr ago, some stone tools were probably being used to create wooden implements. If further studies show that such tools are common at early hominid sites, then the manufacture of implements such as digging sticks and spears may eventually be inferred as part of early adaptive patterns.

The discovery that interpretable patterns of microscopic wear can be preserved on cryptocrystalline silica implements even from sites 1.5 Myr old implies that microwear analysis can be profitably applied to suitable stone artefacts of all ages. We should, therefore, be able to document the successive appearance of activities, such as animal butchery, wood working, hide preparation, bone- and antler-working, throughout the entire Palaeolithic. Such investigations will enable archaeologists to discuss with greater certainty crucial aspects of the behavioural repertoire of early hominids.

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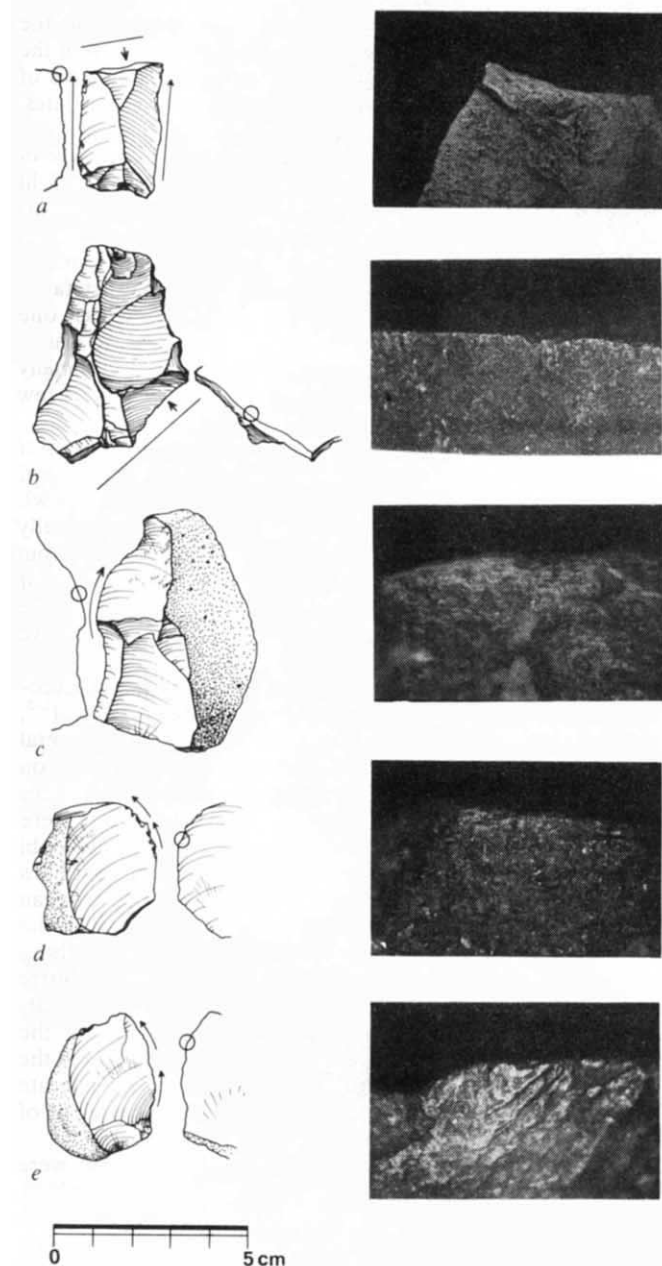


Fig. 1 Illustrations of five used stone artefacts from Koobi Fora accompanied at the right by photomicrographs of their microwear polishes. In the drawings, arrowheads indicate the general direction of striae, adjacent lines show linear extent of microwear traces along an edge and open circles mark where the photomicrographs were taken. Microwear polishes are the lighter, less grainy textured areas in the photomicrographs. All the photomicrographs were taken using an Olympus BHM microscope ($\times 50$); the width of each frame is ~ 0.95 mm. *a*, Plant-cutting knife (18IH-1165) and photo of microwear polish; *b*, wood scraper (18GS-5971) and photo of polish; *c*, knife (of ignimbrite) probably used on meat and/or fresh hide (50-1113) and photo of polish with striae; *d*, meat knife (50-868) with photo of polish (at very edge); *e*, plant-cutting knife (50-1767) and photo of polish.

Evidence for the dilution effect in the selfish herd from fish predation on a marine insect

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It has been proposed that a major factor underlying the evolution of gregarious behaviour is a reduction in the risk of being attacked by predators¹⁻³. One way in which individuals may gain protection from predators by joining a group is through a simple 'dilution' effect—for any one predator attack, the larger the group of prey animals, the smaller is the chance that any particular individual will be the victim. We present here field observations of predation on a marine insect, in which it is possible, for the first time, to quantify the magnitude of the dilution effect and distinguish it from other benefits of group living.

There are two reasons why it has proved difficult to obtain reliable evidence that the grouping of prey animals reduces the risk of predation by a simple dilution effect. First, predation attempts, particularly on vertebrate prey, tend to be relatively rare and it is therefore difficult to quantify the effect. Second, the dilution effect is likely to be masked by other advantages of group living, such as improved efficiency of feeding and reproduction or, more importantly, improved detection and confusion of the predator. This is why, in the lily pond scenario envisaged by Hamilton², the approach of the snake predator is unseen and cannot therefore directly initiate avoidance behaviour by the frogs. The frogs, nevertheless, form into groups and in so doing reduce the individual's chance of being eaten. Several studies have indicated that predator success decreases as the group size of the prey increases (for example, see refs 4-6). However, partly because in these studies the predator's approach was visible to the prey, it is unclear to what extent the benefits of increased group size are the result of a dilution effect or of some other effects, such as improved avoidance behaviour.

We have studied predation by a small (~4 cm) fish (juvenile *Sardinops sagax*: Clupeidae) on a marine insect, the ocean skater *Halobates robustus* (Hemiptera: Gerridae)⁷⁻¹⁰. This system is ideal for a study of predation on the selfish herd and provides a surprisingly close parallel to Hamilton's lily pond phantasy. Groups of readily visible insects were confined in two dimensions on the surface of the sea. The insects were not shielded by vegetation or other natural objects and one would therefore predict that cover-seeking behaviour would be well developed¹. The fish and the insects occurred in very large numbers and predation attempts were frequent. The prey could not see their fish predators under the water surface⁸, therefore group size could not in this respect improve predator detection or enhance avoidance behaviour, such as is initiated by the approach of predators from the air⁸⁻¹⁰. There are two further simplifying factors. Observations can be restricted to animals that are not feeding, as the individuals in flotillas appear not to feed, and solitary, non-hunting individuals can be distinguished on the basis of their behaviour from solitary, hunting ones⁸. In this study, observations were confined to flotillas composed almost entirely of juveniles: complications associated with mate-searching and reproduction are therefore unlikely to be important.

The insects and their predators were observed on the seawater surface close to a lava shore at Caleta Tortuga Negra, Isla Santa Cruz, Galápagos Islands, during September 1980. The clupeid fish swim in shoals of several hundred individuals ~25-50 cm below the water surface. Individual fish hunt *H. robustus* by

swimming swiftly to the surface, pecking briefly at an insect and then rapidly swimming down to rejoin the school.

We observed *Halobates* flotillas of different sizes (including solitary individuals) for periods of 5 min and counted the number of attacks on them. There was a clear relationship between number of attacks per individual per unit time and group size, which on a double log plot gives a straight line with a slope of -1.118 ± 0.123 (95% confidence limits) (Fig. 1). The slope of this observed relation is not significantly different from the expected slope of -1.000 , assuming a simple dilution effect with the probability of attack per individual being inversely related to group size. An alternative way to establish this effect is to test whether the attack rate per group is independent of group size. There is no significant correlation between group size and attack rate per group (Spearman rank correlation coefficient, $r_s = -0.26$, $n = 33$), which supports the idea of a dilution effect.

We also recorded the number of captures per unit time and this revealed a further benefit of group living. The capture rate per group declines with group size ($r_s = -0.66$, $n = 33$): groups of 150 suffer fewer attacks than do solitary individuals ($P < 0.001$, Mann-Whitney U test, $n_1, n_2 = 14, 4$). Therefore, being in a group not only reduces the risk of attack, by the dilution effect, but also reduces the likelihood of the success of such attacks. However, this further benefit is relatively small compared with that conferred by the dilution effect. For example, an individual in a group of 15-17 is about 16 times less likely to be attacked than is a solitary individual (Fig. 1) but the probability of success of such attacks is only three times smaller than for a solitary individual. The mean success of attacks on a solitary individual is $20.5 \pm 3.3\%$ (14), whereas on an individual in a group of 15-17 the mean success is $6.6 \pm 3.8\%$ (14) (mean $\pm 2 \times$ s.e.m. (n)). It is probable that attacks on larger groups are less successful because of greater predator confusion: even when undisturbed, individuals in *Halobates* flotillas are in almost constant motion, making corrections for local water movement and frequently bumping into each other⁷⁻⁹.

This insect and its fish predators provide a natural experiment in which it becomes clear, apparently for the first time, that the individuals in a group can gain precisely the kind of protection from attack that was predicted by Williams¹ and Hamilton² and demonstrated in the laboratory by Milinski¹¹.

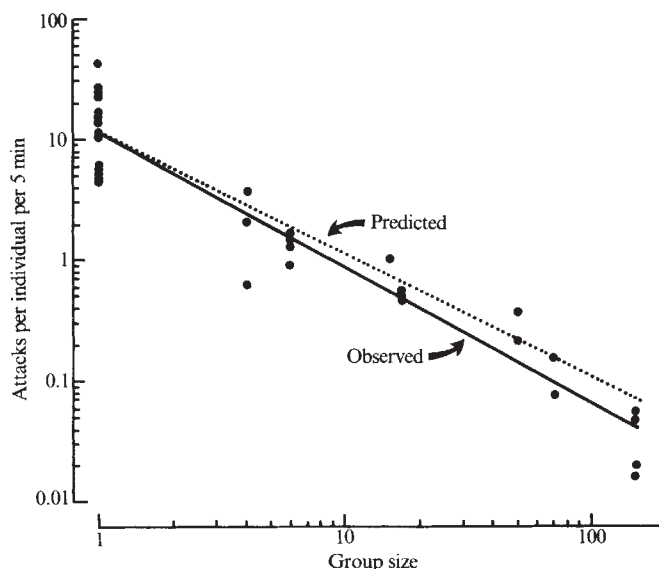


Fig. 1 Relationship between rate of attack per individual *Halobates* and the size of the *Halobates* flotilla. Rate of attack is plotted as the number of attacks in a 5-min observation period, divided by the size of the group. The observed slope (solid line) is -1.118 ± 0.123 (95% confidence limits; $n = 33$). Dotted line represents the expected slope of -1.000 , assuming a simple dilution effect. Observations were made on Isla Santa Cruz, Galápagos Islands, in September 1980.

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Paternal inheritance of a daughterless sex ratio factor

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Here we report an unusual case of extrachromosomal inheritance in the parasitic wasp, *Nasonia vitripennis*. It was accidentally discovered during attempts to select for genetic variability in the sex ratio produced by females of this wasp. The trait, henceforth termed 'Daughterless' (DI), is transferred paternally and causes the mates of carrier males to produce only sons. The effect is not due to mortality of female offspring but rather to an increase in the number of male offspring. DI can be shown to be extrachromosomally inherited by experimental use of the haplodiploid sex determination of this wasp. After introduction at low frequency, the trait increases to predominance in an experimental population within a few generations. The DI trait is of theoretical interest because of its paternal inheritance, and may have practical applications as a biological control agent in pest organisms with haplodiploid sex determination.

N. vitripennis is a small chalcidoid wasp which parasitizes the pupae of various fly species. Typically, 20–40 eggs are laid on to a fleshfly (*Sarcophaga bullata*) host and only 5–15% of these are males. Development from egg to adult takes 14 days at 24 °C. Males and females can be easily sexed at the pupal stage and then isolated for controlled matings. As in other hymenoptera, *N. vitripennis* has haplodiploid sex determination; unfertilized (haploid) eggs develop into males and fertilized (diploid) eggs develop into females. This mode of sex determination gives *N. vitripennis* control over the sex ratio among offspring^{1,2}.

Originally, an experiment was designed to select for genetic variability in the sex ratio produced by females of the wasp. Ten virgin males and females were collected from each of 10 different wild-type stocks of *N. vitripennis* and mated randomly. Five of the stocks had been collected from nature within the previous 2 months. The following regime was used for each generation. Females were isolated singly on hosts for 24 h, then after ~12 days the hosts were opened, sex ratios recorded and

wasp pupae removed. For each generation, 400 female and 200 male pupae were selected and placed in a quart container where eclosion and mating occurred. Females (100–150) were then removed from this container and isolated singly on hosts for 24 h to begin the next generation.

For the first two generations, pupae were selected randomly from all the broods for the next generation. This allowed for repeated interbreeding between the original cultures. On the third generation, 'high' and 'low' sex ratio selection lines were begun. For the high (high proportion of males) line, half of the broods with the highest sex ratios was used to select the 400 female and 200 male pupae for the next generation. For the low line, we used half of the broods with the lowest sex ratios. For the first selection generation, progeny for high and low lines were taken from the mixing population. In subsequent generations, they were genetically isolated from one another (see Fig. 1), with selection occurring in each generation.

It was expected that if there was heritable variability in the sex ratio, then the high line would evolve a high proportion of sons. As the normal sex ratio of the wasp was very low (5–15% sons)², little change in the low line was expected.

Results are shown in Fig. 1. There was a dramatic increase in the sex ratio of the high line, due to the production of all-male broods. By the fourth selection generation there were so few females in the high line that selection had to be relaxed. The production of all-male broods persisted even in the absence of selection, remaining at a frequency of 0.40–0.65 until the ninth generation, when the line was terminated. The occurrence of all-male broods was not due to differential mortality of female offspring. For example, in generation 5 the mean number of progeny in high line all-male broods (25.9 ± 7.8 (s.d.), $n = 54$) was not significantly different from high line mixed-sex ratio (26.9 ± 6.0 , $n = 67$; t test, $P > 0.4$) or low line (27.4 ± 8.2 , $n = 120$; $P > 0.35$) broods. The sex ratio bias is caused by the production of male offspring instead of female offspring.

A series of crosses were done to determine whether the all-male trait was a characteristic of high line males or high line females. High line males and females were crossed to either low line or scarlet-eye (ScDr) stocks³. The all-male brood trait was expressed in crosses involving high line males, indicating that they were the carriers of the trait (see Table 1). This result was rather surprising because haploid males transfer no chromosomal genes to their all-male broods (due to haplodiploidy). Thus, the males are effectively sterile, and it is difficult to select for chromosomal genes for male sterility in a

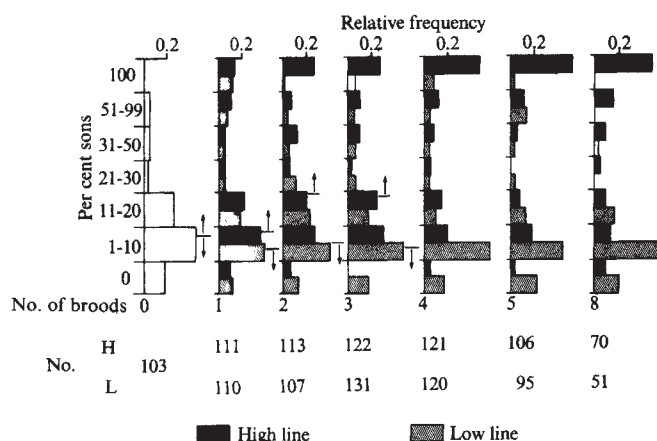


Fig. 1 Selection experiment for high and low brood sex ratios in *N. vitripennis*. The cut-off sex ratios for progeny selected for the next generation are indicated by arrows. Within two generations there was a dramatic increase in the frequency of all-male broods in the high line. The all-male trait persisted in the population after selection was relaxed in the fourth generation.

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Table 1 Crosses between the high line, low line and ScDr demonstrate that the all-male (DI) trait is a characteristic of high line males

Cross $\delta \times \text{♀}$	No. of broods	
	All-male	Mixed
High $\times$ low	33	10
High $\times$ high	22	13
High $\times$ ScDr	52	23
Total	107	46
Low $\times$ low	0	31
Low $\times$ high	3	20
Low $\times$ ScDr	3	63
Total	6	114
ScDr $\times$ low	0	29
ScDr $\times$ high	2	53
ScDr $\times$ ScDr	2	28
Total	4	110

population experiment. Furthermore, such genes should not have persisted once selection was relaxed.

The DI trait was shown to be inherited extrachromosomally by testing all-male brood progeny from the high line male $\times$ ScDr female crosses. As expected, male progeny from the crosses were ScDr in genotype, indicating that an unusual mechanism such as the replacement of the female (ScDr) pronucleus by the high line male pronucleus was not occurring. Therefore, the males did not receive chromosomal genes from the high line. However, 76% of the males received the high line DI trait, producing all-male broods when mated with ScDr females (Fig. 2). By definition, therefore, the DI trait is extrachromosomally inherited through the paternal line. Figure 2 also shows a simultaneous test of virgin high line females to determine whether DI is transmitted to their male offspring. This test revealed no maternal transmission, although it is possible that there was low-level maternal transmission. DI is only the second known example of extrachromosomal inheritance primarily through the male line⁴.

Crosses to three different wild-type stocks have substantiated the paternal extrachromosomal inheritance of DI. It is transferred to each successive generation, with ~75% of the males in any all-male brood inheriting the trait. DI males readily mate; they have (normal) haploid spermatogonia and produce motile sperm which are transferred to the female spermatheca during copulation.

To determine whether the trait would increase in the absence of selection, DI carrier males were introduced into an experimental population at a frequency of 0.05. The same design was used as in the previous population experiment, but without selection for brood sex ratio. The DI trait increased to a

frequency of 0.63 in two generations and remained at about that level for two more generations, until the line was terminated. Thus the trait spread rapidly through a randomly mating population in the absence of any experimentally induced selection.

The causative agent of the DI trait is unknown. DI could be a cytoplasmic factor located within the spermatozoa, which interferes with incorporation of the male pronucleus after fertilization has occurred. However, transmission electron microscopy has so far revealed no unusual cytoplasmic particles associated with spermiogenesis in carrier males. Alternatively, DI may be an extracellular infectious agent which is transferred to the female reproductive tract during copulation. There it could interfere with the fertilization mechanism and be transmitted to ova passing down the reproductive tract. These possibilities are being investigated.

In many organisms, virus-, rickettsia- and spirochaete-like factors are known to be cytoplasmically transmitted through the maternal line⁴⁻⁸. Some of these cause a female-biased sex ratio, either by mortality of male offspring^{7,8} or by affecting sex determination^{9,10}. Population genetic theory predicts that maternally transmitted factors which bias sex ratio towards female offspring will increase in frequency in many circumstances^{5,6}. Similarly, paternally transmitted factors should bias the sex ratio towards male offspring. DI is the first paternally transmitted extrachromosomal factor discovered which causes a sex ratio bias, and it is consistent with theory. By producing all-male broods, a DI factor increases its frequency of paternal transmittance and will spread rapidly through an outbreeding population, as shown experimentally. The demic population structure of *N. vitripennis* in nature probably limits the increase of DI in this species². For example, some host pupae are highly dispersed such that the (flightless) males can only mate with sisters. A DI factor producing only males is eliminated in these broods because the males have no mates.

Haplodiploid sex determination presents unique opportunities for paternal extrachromosomal inheritance. In diploid organisms, a paternal extrachromosomal factor which causes effective male sterility will not be transmitted to the progeny because there will be few, if any, viable progeny to inherit the trait. In contrast, females of many haplodiploid organisms who mate with effectively sterile males can still produce (haploid) male progeny. This presents an 'evolutionary opportunity' for paternal transmission. We therefore predict that DI factors will be found in many haplodiploid species. Evidence supporting this is the occurrence of colony extinctions due to the production of all-male broods in laboratory rearing of parasitic wasps¹¹. Such occurrences may be due to the form of genetic sex determination¹², but DI factors may also be responsible. Whether DI occurs in a particular species will depend on the population structure of that species, its normal sex ratio and the nature of transmission of the factor.

A DI factor introduced into a pest haplodiploid species may persist for many generations and could drive the population to extinction or maintain it at low levels. Thus DI factors may be of value in controlling haplodiploid pests such as sawflies, seed chalcids and certain phytophagous mites.

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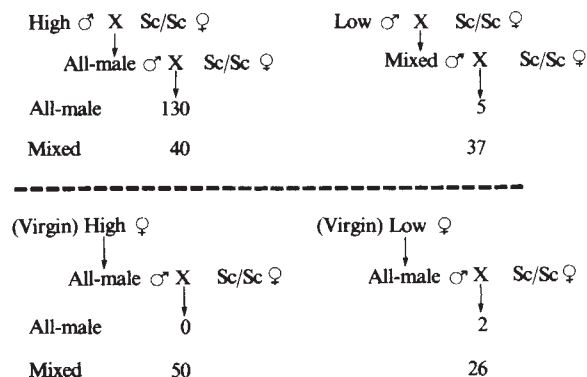


Fig. 2 A test for the inheritance of the high line DI trait demonstrates that it is transmitted from high line males to the male progeny of their mates. High line females do not transmit the trait to their male progeny. Sc/Sc indicates ScDr genotype females.

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Geometry of neonatal neurones and the regulation of synapse elimination

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In the ciliary ganglion of adult rabbits, ganglion cells lacking dendrites are generally innervated by a single axon, whereas cells with one or more dendrites are innervated by a number of different axons that increases in proportion to the complexity of their dendritic arbor¹ (see also refs 2–4). We have now explored the basis of this correlation by comparing the dendritic arborizations of cells receiving different numbers of axons during and after the period of synapse elimination that occurs early in postnatal life⁵ (see also ref. 6). Our results suggest that the geometry of neonatal neurones influences the competitive interaction between the several axons that initially innervate the same cell. This finding in turn implies that an important function of dendrites is to regulate the number of different axons that ultimately innervate each neurone.

Neonatal rabbits (New Zealand white, 1–7 days old) were anaesthetized with pentobarbitone. Both ciliary ganglia were removed, placed in a Plexiglas chamber and continuously superfused at room temperature with oxygenated mammalian Ringer fluid⁵. The oculomotor nerve, which carries the pre-ganglionic axons, was drawn into a suction electrode for stimulation. Ganglion cells were impaled with glass microelectrodes filled with 4% horseradish peroxidase (HRP; Sigma type VI) in a solution of 0.2 M potassium acetate buffered to pH 7.6 ($R = 60$ – $100\text{ M}\Omega$). The number of different axons innervating each impaled ganglion cell was estimated by counting the increments in the postsynaptic response as the strength of the stimulus to the oculomotor nerve was gradually increased; the details and limitations of this method are given elsewhere^{1,2,5}. HRP was then injected into the cells (one to three neurones per ganglion) either by iontophoresis or by pressure; both methods gave similar results. The ganglia containing the HRP-filled neurones were processed according to the technique of Hanker *et al.*⁷ and examined in whole mounts. *Camera lucida* drawings were made of the successfully injected cells. Our criterion of acceptable staining was that each labelled cell have an axon easily visible for at least $200\text{ }\mu\text{m}$ from the cell body; most axons could be followed much farther. As in a previous study¹, a primary dendrite was defined as a tapered process, other than the axon, that extended at least $5\text{ }\mu\text{m}$ radially from the cell body. For quantitative analysis of neuronal geometry, the labelled neurones and their processes were traced in three dimensions with a microscope/computer system⁸. These data were compared with results obtained from ganglion cells in adult rabbits (3–5 months old) studied with the same methods¹.

The number of preganglionic axons that innervated each of 313 neurones in neonatal ganglia was determined electrophysiologically. As previously shown⁵, neonatal neurones were, on average, innervated by about twice as many different axons as cells in ganglia from adult rabbits (Fig. 1). Despite this difference, the observed range of the number of axons that innervated individual ganglion cells (one to seven axons) did not change during postnatal development. This suggests that there is little or no postnatal reduction in the number of axons that initially innervate some neonatal cells, whereas other neurones lose the synapses from most of the axons that contacted them at birth. Although this result might to some degree reflect a limitation of our technique for counting the number of innervating axons, it implies that the competitive elimination of

initial inputs proceeds more vigorously on some ganglion cells than on others.

Of the 313 neonatal ganglion cells studied electrophysiologically, 110 were successfully stained with HRP. Some of these neurones lacked dendrites altogether, some had relatively modest arborizations, while others had extensive dendritic arbors (Fig. 2). This range of dendritic complexity resembles that found in adult ganglia¹. However, the distribution of cells within this range was somewhat different early in development. There were fewer neonatal cells that lacked dendrites entirely and more cells with five or more primary dendrites (Fig. 3). Moreover, the dendritic arborizations of neonatal neurones had more branch points on average than did their adult counterparts (see Fig. 3 legend). Thus, assuming that the cells sampled in newborn and adult animals were representative, there is a modest postnatal remodelling of dendritic arborizations.

Despite these quantitative differences between neonatal and adult arborizations, our results show that most neonatal ganglion cells possess dendrites at birth, several weeks before synapse elimination is complete⁵ and, furthermore, that some ganglion cells lack dendrites at birth. Synapse elimination therefore occurs within a population of ganglion cells that represents the full range of adult geometries.

Although the adult range of neuronal geometries is present at birth, the correlation seen in adult ganglia between number of inputs and dendritic complexity was not apparent in neonatal ganglia (Fig. 4). Thus neonatal ganglion cells that lacked dendrites altogether were innervated by about as many different axons as the most geometrically complex cells in our series. In ganglia from adult animals, on the other hand, the most complex cells were innervated by about five times as many axons as the cells without dendrites¹. Therefore the matching of geometry and innervation occurs postnatally. Moreover, the approximately equal numbers of inputs to cells with and without dendrites in neonatal ganglia suggest a disproportionate loss of inputs from those neonatal neurones that have few or no dendrites at birth.

It is generally recognized that the number of different axons that innervate neurones is a major determinant of their ability to integrate information. In several different parts of the nervous system, this number seems to be set postnatally when the elimination of some synaptic connections reduces the number of axons that initially innervate each target cell⁶. The nature of the competitive interaction underlying this process is not well

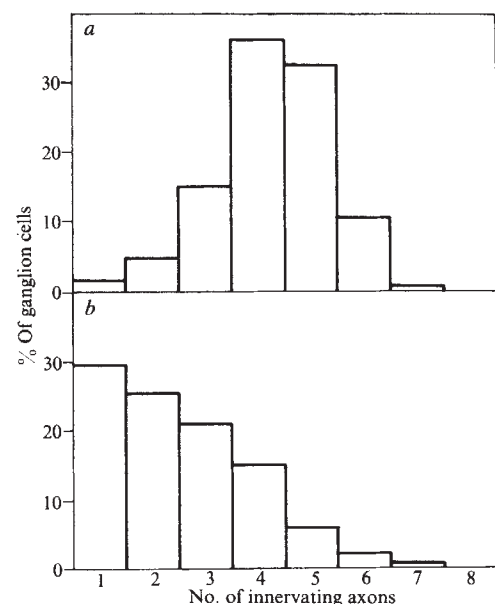


Fig. 1 Proportion of ganglion cells innervated by different numbers of preganglionic axons in neonatal (a) and adult (b) ganglia. $n = 313$ (a) and 152 (b).

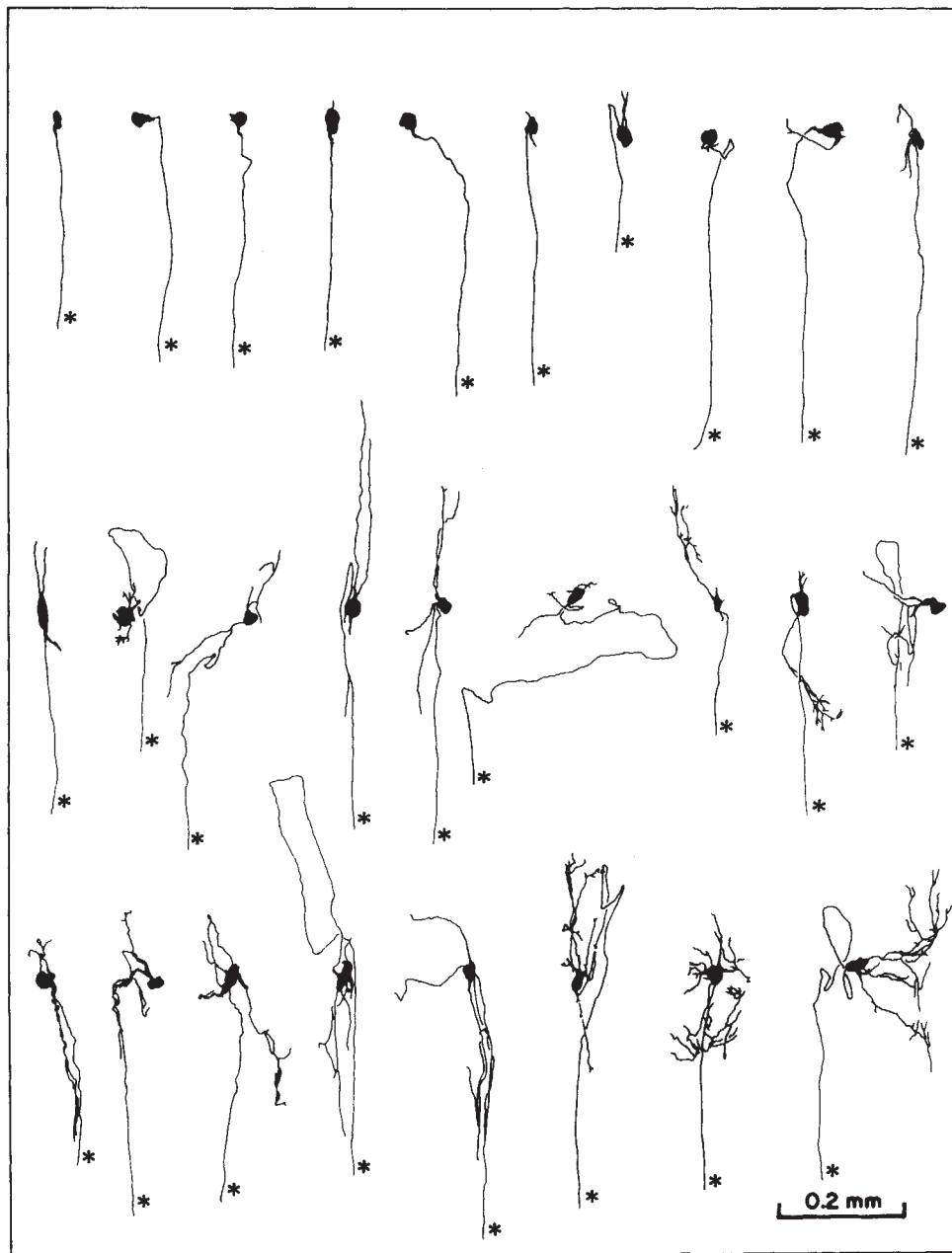
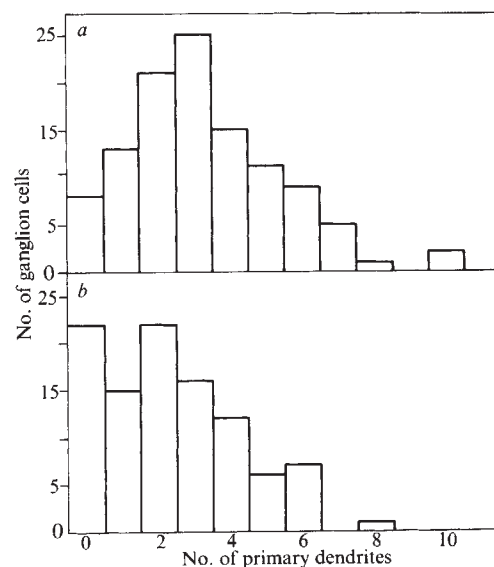


Fig. 2 Camera lucida drawings of neonatal rabbit ciliary ganglion cells. We arbitrarily chose for illustration every fourth cell in the series of labelled cells that we examined; neurones are arranged in order of increasing complexity to emphasize the range of neuronal geometries. The axon of each cell is indicated by an asterisk.

Fig. 3 Distribution of the number of ciliary ganglion cells having various numbers of primary dendrites in neonatal (*a*) and adult (*b*) animals. As the number of cells studied in neonates and adults was similar ($a = 110$, $b = 101$), these graphs can be compared directly. The mean number of primary dendrites was greater for neonatal than adult neurones (mean $\pm$ s.e. = 3.3 ± 0.2 compared with 2.3 ± 0.2). In addition, the dendritic arborizations of neonatal neurones had more total branch points than adult neurones (6.4 ± 0.8 compared with 3.1 ± 0.6). Despite the greater complexity of neonatal arborizations, the summed length of all dendritic segments was about the same in neonatal and adult neurones ($594 \pm 50 \mu\text{m}$ compared with 600 ± 74). This is because the length of individual primary dendrites in neonates (including subsidiary branches) was on average less than the adult length (neonatal length = $181 \pm 10 \mu\text{m}$, $n = 362$; adult length = $258 \pm 19 \mu\text{m}$, $n = 235$).



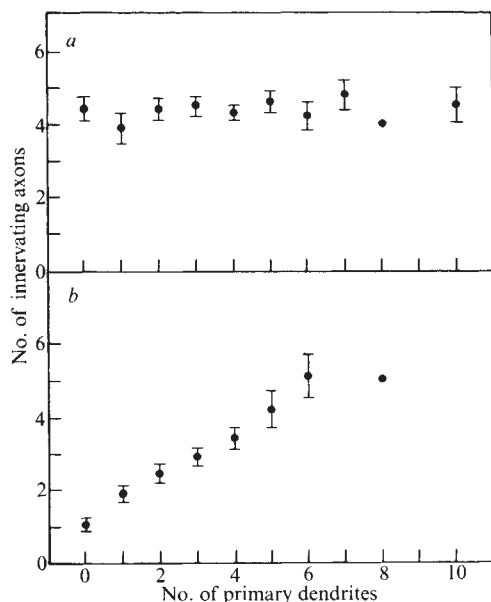


Fig. 4 Relationship between the number of innervating axons and the number of primary dendrites possessed by ganglion cells in neonatal (a) and adult (b) animals. $n = 110$ (a) and 101 (b). Bars represent standard errors. b Is redrawn from ref. 1.

understood. Our aim here was to explore the possibility that the competition between axons initially innervating the same cell is influenced by the geometry of the postsynaptic neurone. The major results relevant to this issue are that ganglion cells in newborn animals display the full range of adult geometries, even though virtually every neonatal neurone is innervated by several axons. As a few weeks later each cell is innervated by a number of axons that is proportional to the complexity of its dendritic arbor, these observations imply that geometry somehow regulates the competitive interaction between axons.

We have not ruled out other possible explanations of the adult correlation between inputs and geometry. For example, the number of innervating axons and the complexity of dendritic arbors might be independently controlled by a third factor. Furthermore, we have assumed that adult geometries arise from gradual and minor alterations in the similar geometries of neonatal neurones. It is possible, however, that ganglion cells undergo drastic changes in which neonatal neurones without any dendrites acquire them, and those with complex dendritic arbors lose them entirely.

Given that these possibilities are unlikely, the simplest explanation of our findings is that competition between axons takes account of the pre-existing geometries of the postsynaptic ganglion cells. In this way the neonatal pattern of innervation, in which there is little or no correlation between the number of inputs and postsynaptic geometry, would be transformed into the adult pattern in which inputs and geometry are highly correlated. Because mature cells without dendrites are generally innervated by the terminals of a single axon, a paucity of dendrites at birth evidently forces the elimination of all but a single set of axonal endings. Conversely, the presence of dendrites confers some degree of protection against competitive interaction; cells with dendrites remain innervated by a number of different axons that is proportional to their geometrical complexity. To the extent that competition is influenced by postsynaptic geometry, the dendritic remodelling that occurs postnatally (see Fig. 3) would also affect the outcome of synapse elimination.

Thus competition between axons seems to be fostered by confinement of the terminals of several axons to a limited postsynaptic surface, and mitigated when these synapses are

deployed over a more extensive postsynaptic geometry. The influence of geometry on competition seems unlikely to be based on the availability of synaptic sites because the number of synapses actually increases during synapse elimination^{2,5} (see also refs 3,4). Evidently there is ample space for additional synapses, as long as they arise from the axon or axons ultimately destined to innervate the neurone in question. Perhaps complex postsynaptic geometries mitigate competition by allowing the sets of synapses arising from different axons to be spatially or electrotonically segregated from one another.

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Single channel recordings of Ca^{2+} -activated K^{+} currents in rat muscle cell culture

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Many nerve and muscle cells possess a Ca^{2+} -activated K^{+} conductance which gives rise to a long-lasting hyperpolarization following Ca^{2+} entry during an action potential and which appears different from the classical Hodgkin and Huxley voltage-activated K^{+} conductance^{1–7}. Using the extracellular patch clamp technique^{8–12} to record the currents from patches of intact and isolated plasma cell membrane from rat myotubes, we have observed single channel currents that would give rise to this Ca^{2+} -activated K^{+} conductance. The channels are highly selective to K^{+} and have a conductance near 100 pS at physiological $[\text{K}^{+}]$, a value 10–20 times greater than that of the classical voltage-activated K^{+} channels. The frequency with which these channels open and effective open times are controlled in a reversible manner by the free $[\text{Ca}^{2+}]$ on the intracellular side of the membrane; the channel seldom opens when the $[\text{Ca}^{2+}]_i$ is $<10^{-8}$ M, but opens with increasing frequency as the $[\text{Ca}^{2+}]_i$ exceeds 5×10^{-7} M. With $0.5 \mu\text{M}$ Ca^{2+}_i , both opening frequency and effective open times depend on membrane potential, increasing as the intracellular side of the membrane is made more positive. At higher levels of Ca^{2+}_i (50 μM), the channel is almost continuously active, even at hyperpolarized membrane potentials.

Figure 1 shows single channel currents recorded from intact myotubes for three different membrane potentials of the patch. A fire-polished micropipette (3–10 M Ω) was sealed ($>10^9 \Omega$ seal) against the surface membrane by applying suction. Once sealed, the potential of the membrane patch was clamped to the desired level by polarizing the electrode with respect to ground,

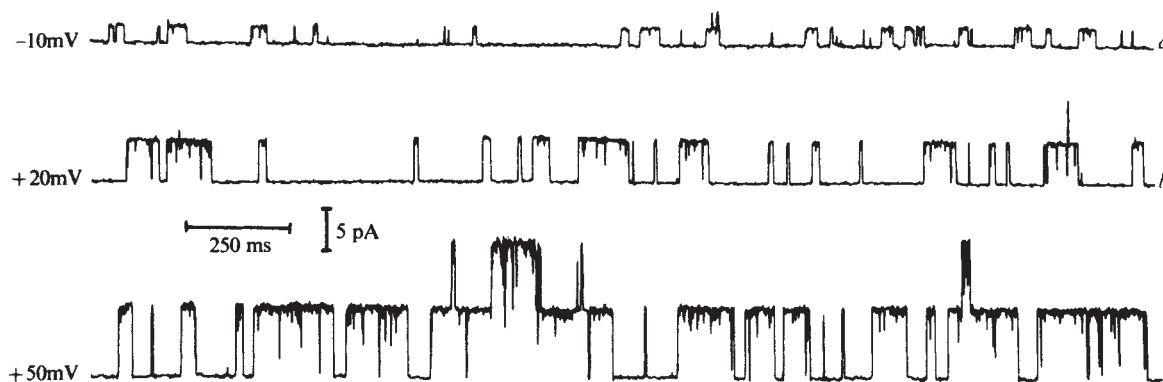


Fig. 1 Single-channel currents recorded from a patch of membrane on an intact myotube at three different membrane potentials. The exterior surface of the membrane patch was clamped to -40 mV (a), -70 mV (b), and -100 mV (c) with the patch electrode; assuming a resting potential of -50 mV, the membrane potential of the patch was then -10 , $+20$ and $+50$ mV. Active filtering with high frequency cutoff at 1 kHz (18 dB per octave) was used. The extracellular solution and the solution in the pipette consisted of (mM): KCl, 4 ; NaCl, 155 ; CaCl_2 , 2 ; MgCl_2 , 2 ; TES (N-tris(hydroxymethyl)-methyl-2-aminoethane sulphonic acid), 2 ; glucose, 11 ; pH 7.0 . Temperature 20°C . Channels of this type were active in less than 10% of the membrane patches on intact cells, and then only during applied depolarization; they were active in over 90% of the excised membrane patches when the intracellular surface of the membrane was exposed to Ca^{2+} (Figs 2, 3, 4). These results suggest that the normal intracellular $[\text{Ca}^{2+}]$ in the intact myotubes in our experiments was typically less than that required to activate the channels (5×10^{-7} M). The flickering of open channels towards the closed state suggests that the channel may fluctuate rapidly between an open state and a transient intermediate closed-channel state¹³. Acetylcholine in the pipette (0.5 μM) would not activate this channel, but would activate the acetylcholine receptor channel which has a lower conductance and a reversal potential near 0 mV^{10,11}. With acetylcholine in the pipette, both types of channels were often active in the same membrane patch.

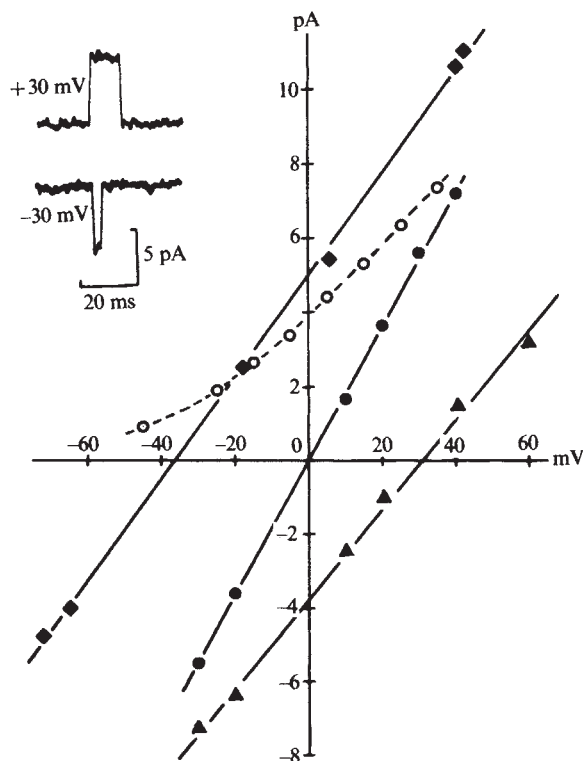


Fig. 2 Single-channel current amplitude as a function of membrane potential. Open circles, data from a membrane patch on an intact cell plotted assuming a resting potential of -50 mV; solutions and experimental procedure as in Fig. 1 but different membrane patch. Inset, single channel currents recorded from an excised membrane patch clamped at $+30$ and -30 mV. The solutions bathing both sides of the membrane patch were identical (mM: KCl, 144 ; NaCl, 16 ; MgCl_2 , 2 ; TES, 2 ; glucose, 11 ; pH 7.0), except that free $[\text{Ca}^{2+}]$ on the extracellular side was 10^{-8} M (2 μM total Ca^{2+} and 40 μM EGTA added to the solution), and free $[\text{Ca}^{2+}]$ on the intracellular side was 0.5 μM (10 μM total Ca^{2+} and 14 μM EGTA). ●, Single channel current amplitudes recorded from this patch at a series of membrane potentials. Maintaining the extracellular KCl at 144 mM, reducing the intracellular KCl to 32 mM, and adding glucose to maintain osmolarity gave the data plotted as ▲. Maintaining the intracellular KCl at 144 mM, reducing the extracellular KCl to 32 mM and increasing extracellular NaCl to 128 mM gave the data plotted as ◆.

and currents from the patch were recorded¹⁰⁻¹². The rectangular shape of the single channel currents suggests that the observed channel opens and closes in an all-or-none manner similar to other membrane channels⁸⁻¹³. Once open, the channel either closed after a few milliseconds, or remained predominantly open while flickering towards the closed channel state until finally closing 10 – 300 ms later. We could not establish whether this flickering was due to partial channel closures or to brief but complete closures, since the resolution of very brief events was limited by the frequency response of the recording system (time constant, 400 μs). As the patch was polarized to $+50$ mV (inside positive), the currents became larger and the channel usually flickered for longer periods before finally closing, increasing its effective open times. Occasionally, a second channel would open, stepping the current to twice that observed with a single open channel.

In the conditions of the experiment in Fig. 1, the observed single channel currents could arise from an efflux of K^+ from the myotube and/or an influx of Cl^- . Na^+ could not be a major charge carrier as the flow of Na^+ down its electrochemical gradient would produce single channel currents with a polarity opposite to that observed. To determine whether K^+ or Cl^- carried the channel current we changed the concentrations of ions on one or both sides of excised membrane patches, obtained by pulling the pipette with its associated membrane patch away from the cell^{10,13}; the solution in the pipette then bathed the extracellular side of the excised membrane patch and the bath solution bathed the intracellular side.

The solid circles in Fig. 2 plot single channel current magnitudes as a function of membrane potential for a membrane patch in which the concentration of KCl (144 mM) was identical on both sides of the membrane. The single channel conductance, calculated from the slope of the line through the filled circles, was 187 pS. The reversal potential was about 0 mV, as expected with essentially identical solutions on both sides of the membrane.

144 mM KCl in the pipette and 32 mM KCl bathing the intracellular surface of the membrane patch shifted the reversal potential to $+31$ mV (solid triangles). Reversing the ionic gradients shifted the reversal potential to -36 mV (solid diamonds). In both of these cases the single channel conductance was about 130 pS. Total replacement of the Cl^- with SO_4^{2-} or 2 (N-morpholino) ethane sulphonic acid (MES) had little effect on the conductance or reversal potential of the channel. The shift in equilibrium potential in these and addi-

tional experiments was in general agreement with that predicted by the Nernst equation for a K^+ -selective channel, suggesting that in the conditions of these experiments the primary charge carrier through this channel is K^+ . Such high selectivity is interesting as the conductance of this channel is about three times that reported for the less selective acetylcholine-activated channel in the same preparation^{10,11}.

The single-channel conductance was independent of membrane potential (+60 to -60 mV) when $[K^+]$ was 32 mM or higher on both sides of the excised membrane. However, in the intact cell bathed in 4 mM K^+ single channel conductance appeared to decrease near the reversal potential; single channel (slope) conductance ranged from 60 pS near the resting potential (-50 mV) to 100 pS when the membrane patch was depolarized to 0 mV (Fig. 2, open circles).

To determine if the K^+ channels shown in Figs 1 and 2 are Ca^{2+} activated we examined the effect of $[Ca^{2+}]$ at the inner

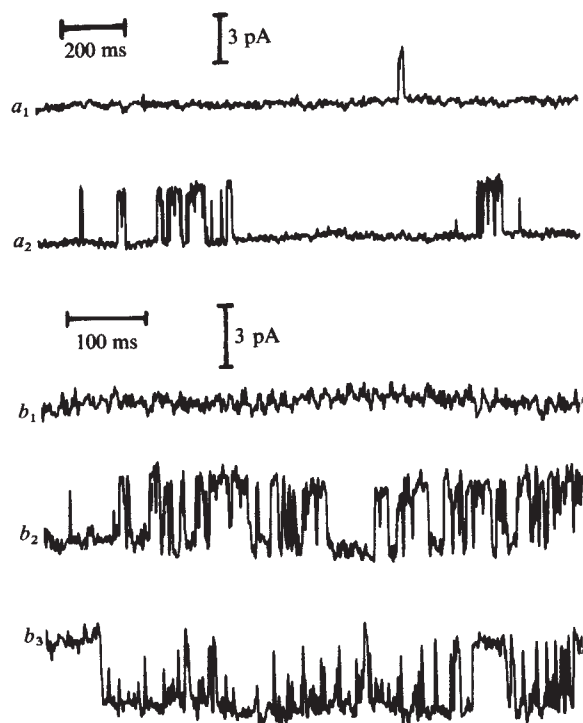


Fig. 3 Activation of the channel by Ca^{2+} on the intracellular surface of excised membrane patches. a_1 , Recordings from an excised membrane patch. The extracellular solution contained (mM): KCl, 4; NaCl, 155; $MgCl_2$, 2; TES, 2; glucose, 11; pH 7.0. Both solutions also contained 2 μM $CaCl_2$ and 40 μM EGTA yielding a free $[Ca^{2+}]$ of 10^{-8} M. With these solutions the channel opened about once every 3 s. a_2 , Replacing the intracellular solution with one containing 0.5 μM free Ca^{2+} (10 μM Ca^{2+} and 14 μM EGTA) greatly increased the channel opening rate and effective open times. Membrane potential 0 mV. b_1 , Records from an excised membrane patch (+24 mV) in which the free extra- and intracellular $[Ca^{2+}]$ was buffered to less than 10^{-8} M. No channel activity was observed over a 2-min period while the membrane potential was repeatedly changed over the range -60 to +60 mV. b_2 , b_3 , Increasing the free $[Ca^{2+}]$ on the intracellular side of the membrane to 50 μM led to repeated channel openings at all membrane potentials examined (-60 to +60 mV). Data at +24 mV (b_2 , upward-going single channel currents) and at -27 mV (b_3 , downward-going single channel currents) are presented. Continuous channel activity of this type, interrupted with occasional channel closings lasting longer than several hundred milliseconds, was observed throughout the 10-min exposure to 50 μM Ca^{2+} . In addition to the $[Ca^{2+}]$ listed above, solutions on both sides of the membrane for b also contained (mM): KCl, 144; NaCl, 16; $MgCl_2$, 2; PIPES, 2; pH 7.2.

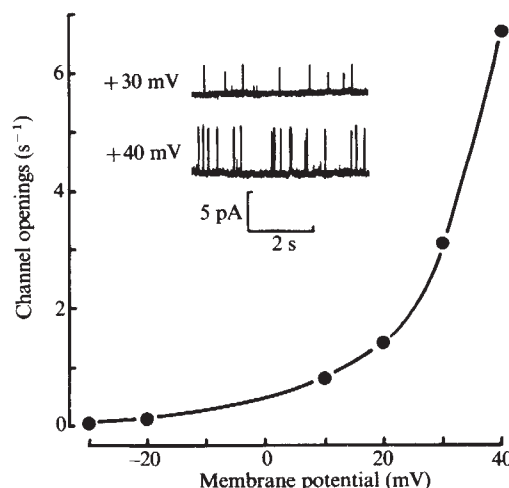


Fig. 4 Effect of membrane potential on the frequency of channel opening for an isolated membrane patch with 0.5 μM free Ca^{2+} on the intracellular side of the membrane. Depolarization to positive holding potentials increased both the frequency of channel opening and the effective open times. The inset shows sample records at +30 and +40 mV. Not all channel openings can be distinguished in these traces since they are displayed on a slow time scale. Both the intra- and extracellular solutions contained (mM): KCl, 144; NaCl, 16; $MgCl_2$, 2; TES, 2; glucose, 11; pH 7.0. In addition, the extracellular solution contained 10^{-8} M free Ca^{2+} (2 μM Ca^{2+} and 40 μM EGTA) and the intracellular solution contained 0.5 μM free Ca^{2+} (10 μM Ca^{2+} and 14 μM EGTA). At higher $[Ca^{2+}]$ (5–50 μM) on the intracellular side of the membrane, membrane potential had less of an effect; the channels were open much of the time.

surface of the membrane using excised membrane patches. When the free $[Ca^{2+}]$ was 10^{-8} M or less, the channel opened only infrequently and then only when the membrane was depolarized near 0 mV or held at positive membrane potentials (Fig. 3 a_1 , b_1). Increasing free $[Ca^{2+}]$ at the inner surface of the membrane to as little as 0.5 μM increased both the number of openings and the effective open times so that the channel typically stayed open 10–20% of the time at 0 mV (Fig. 3 a_2). The Ca^{2+} -sensitivity of this channel is thus similar to the Ca^{2+} -activated K^+ conductance¹⁴. Channel opening did not require Ca^{2+} on the extracellular side of the membrane suggesting that cytoplasmic components other than Ca^{2+} that would be washed from these excised patches are apparently not required for activation.

With 0.5 μM free Ca^{2+} at the intracellular membrane surface the frequency of channel opening and effective open times were an exponential function of membrane potential, increasing as the potential was depolarized or made positive on the intracellular membrane surface (Fig. 4). This effect of membrane potential was reversed by stepping to negative membrane potentials. With 50 μM Ca^{2+} at the intracellular membrane surface, membrane potential had less of an effect on channel kinetics; the channel opened and shut rapidly, spending most of its time in the open state at all membrane potentials examined (+60 to -60 mV, Fig. 3 b_2 , b_3). Ca^{2+} -activation of the channel was reversible by reducing free $[Ca^{2+}]$ at the inner surface of the membrane to less than 10^{-8} M.

The findings above describe a membrane channel with properties that are consistent with the characteristics of the Ca^{2+} -activated K^+ conductance. The channel is mainly permeable to K^+ , is reversibly activated by low concentrations of Ca^{2+} at the intracellular surface of the membrane, and is modulated by membrane voltage in the presence of Ca^{2+} . The single channel conductance of this Ca^{2+} -activated K^+ channel is 10–20 times greater than values that have been reported for the classical (Hodgkin-Huxley) voltage-activated K^+ channel^{13,15,16}.

After this paper was submitted a report appeared¹⁷ describing a Ca^{2+} -dependent channel in chromaffin cell membranes with similar conductance to the channel described here. Further studies are needed to determine whether the ionic selectivity and kinetic properties of these channels are the same.

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The sea anemone *Actinia equina* tolerates allogeneic juveniles but alters their phenotype

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Actinia equina is a variably coloured sea anemone that broods juveniles within its body cavity. Male, female and non-sexual individuals can produce juveniles, even after periods of isolation, and it has been suggested that this species is capable of reproducing by asexual or parthenogenetic means. Further evidence in favour of this hypothesis has been derived from similarities in coloration and in enzyme banding, as shown by electrophoresis, between offspring and their parents^{1–3}. *A. equina* does, however, produce gametes that are morphologically similar to those of other sexually reproducing anemones, which suggests that sexual reproduction must also occur^{1–4}. Thus far the only direct evidence of sexual reproduction has been the occurrence of adults containing differently coloured juveniles within their body cavity; however, this is unusual and indeed in some areas adults only contain juveniles of the same colour as themselves^{1,2,5,6}. Carter and Thorp¹ have suggested that adults can select against sexually produced juveniles that are phenotypically different from themselves; as a result juveniles generally match their parents in coloration. We show here by means of fostering experiments that adults will brood allogeneic juveniles. Surprisingly, juveniles may change colour to match that of their foster parent.

Red *A. equina* used here were from western Scotland (Millport) and had a pale pinkish-red pedal disk. Green individuals, characterized by a light green pedal disk, were obtained from southern England (Roedean). Anemones were maintained in large aquaria (~600 l) containing artificial seawater (Tropic Marin Neu; relative density 1.025) at 19°C; they were not fed during experiments. Five juveniles were transplanted into

each adult as follows. Adults were placed in anaesthetic solution (1 vol seawater: 1 vol 0.6 M MgCl_2) for 10–20 min and then a vertical incision was made in the body wall. Juveniles were flushed from the gastric cavity of the parent by means of a syringe containing seawater. Using a 0.5-mm diameter needle, juveniles were threaded onto a braided polyester suture which was subsequently tied into a small loop. Threaded juveniles were then inserted in the gastric cavity of a foster parent through the incision in the body wall and the looped suture attached to the endoderm of the column. The foster parent was replaced in seawater and the incision healed within 3–4 days. After 8 or 21 days the anemone was again anaesthetized, a further incision made and the suture with attached juveniles removed. Use of this method ensured that the juveniles which recovered at the end of the experiment were those that had been transplanted rather than the foster parent's own offspring. Juvenile coloration was classified into four discrete categories: green, reddish-green, greenish-red and red.

Over 200 green juveniles were removed from ~20 similarly coloured adults; there were no juveniles with red pigmentation. Of these juveniles, 121 were maintained for 21 days attached to the aquarium substrate; as can be seen from Table 1, none of these showed any significant colour change. A further 35 green juveniles were transplanted into 8 green foster parents; 10 of these were recovered after 8 days and 13 after 21 days, and all were still green (Table 1). Eighty green juveniles were transplanted into 13 red foster parents; after 8 days, 10 out of 15 recovered showed some degree of red pigmentation and after 21 days, 18 out of 19 recovered were red to some extent, with 11 of a similar colour to their foster parents (Table 1). Note that there was considerable variation in the extent to which green juveniles transplanted into the same red anemone were affected: sometimes the transplanted brood would contain individuals whose colour was unchanged as well as others whose colour had changed to that of the foster parent. There was some evidence that direct access of the juvenile's external surface to the parent's gastric cavity was one of the factors involved in colour change; in one case two juveniles that were pressed close together on the suture for 21 days became uniformly red apart from the small area of contact between them, which remained green.

The converse experiment was carried out using red juveniles, albeit on a lesser scale owing to the unexpectedly low number of offspring that could be obtained from red adults. Red juveniles that were maintained for 21 days attached to the aquarium substrate or inside red foster parents did not change colour (see Table 1). Of 25 juveniles transplanted into green adults, 16 were recovered after 21 days; only two of these showed any degree of green pigmentation, the remainder being red (Table 1). These do not show any marked tendency of red juveniles to become green when placed in green adults, which contrasts with the clear effect of red adults on green juveniles.

Carter and Thorp¹ have proposed that adults might be able to select against juveniles that are phenotypically different from themselves. This possibility was tested by examining the survival of red juveniles transplanted into green adults and green juveniles transplanted into red adults. All 15 juveniles transplanted for 8 days into differently coloured parents were retrieved apparently unharmed. Of those transplanted for 21 days, 35 out of 55 (64%) were retrieved still attached to the sutures. In addition, eight juveniles with scarred columns (not included in Table 1) were found inside the foster parents and these were presumed to have detached from the sutures during the experiment. This suggests that at least 78% of the juveniles survived 21 days of transplantation into differently coloured parents. Given that some juveniles may have died as a result of the experimental treatment or may have become detached and remained undetected within the adult, these results do not provide any clear evidence of anemones being able to select against phenotypically different juveniles.

We suggest that in natural conditions anemones will tolerate allogeneic juveniles within their body cavity; similarities in

* Deceased.

Table 1 Effects of foster parents on the coloration of juvenile *Actina equina*

Colour of foster parents	Initial colour of juveniles	Final colour of juveniles			Length of experiment (days)
		Green	Reddish-green	Greenish-red	
Control	Green	121	0	0	21
Green	Green	10	0	0	8
Green	Green	13	0	0	21
Red	Green	5	2	4	8
Red	Green	1	3	4	21
Control	Red	0	0	0	21
Red	Red	0	0	6	21
Green	Red	0	0	14	21

Control juveniles were free-living and attached to the aquarium substrate.

coloration may result in part from a direct effect of the adult on its brood. The persistence of this effect is being investigated; preliminary evidence indicates that red pigmentation induced by a foster parent can be retained for at least 2 months after removal from the adult.

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Polyamine starvation causes disappearance of actin filaments and microtubules in polyamine-auxotrophic CHO cells

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The polyamines putrescine, spermidine and spermine are found in all cells and their synthesis increases markedly during cell proliferation^{1–3}. However, their precise physiological role in cellular metabolism is not well understood. A potentially fruitful approach to this problem would be isolation and study of cellular mutants unable to synthesize polyamines. Such mutants have been obtained from bacteria^{3,4} and yeast^{5,6} but, to our knowledge, not from higher organisms. We report here the isolation of a polyamine-deficient variant from Chinese hamster ovary cells that, unlike the corresponding polyamine-depleted *Escherichia coli* mutant⁴, is absolutely dependent on polyamines for continuous replication. We show further that omission of polyamines from the growth medium of these cells causes bundles of actin filaments and microtubules to disappear but does not affect the pattern of the intermediate filaments.

The wild-type Chinese hamster ovary cell line CHO (obtained from the American type culture collection) was routinely cultured in the presence of 5×10^{-7} M putrescine and 10% fetal calf serum. The strain was adapted to grow without serum by culturing in a medium composed of Eagle's minimal essential medium (MEM) and Ham's nutrient mixture F12 in ratio 1:1, supplemented with 0.1% bovine serum albumin, giving a final putrescine concentration of 5×10^{-7} M, while the serum concentration was gradually diminished. Finally a cell strain emerged that grew well without serum but proved dependent on polyamines for growth. This cell strain has now been cultured for 6 months and for more than 100 cell doublings on plastic Petri dishes coated with commercial gelatin composed of pig skin collagen. For subculture the cells were detached with trypsin.

If putrescine was omitted from the growth medium, cellular replication gradually slowed down and ceased in 8–10 days (Fig. 1). However, many of the cells still remained attached to the plates, preserving their normal morphology under the light microscope, although in subsequent passages they did not attach to the plates. In the absence of putrescine, DNA synthesis dropped concomitantly with cellular replication (Fig. 2). If putrescine was added to the cultures after 2 days of putrescine starvation the cells recovered rapidly and completely, but after longer starvation the recovery was slow or absent (results not shown). Cell growth was maintained equally well in medium in which putrescine was replaced by the higher polyamines, spermidine or spermine, in equivalent concentrations (results not shown).

Omission of putrescine from the culture medium depleted the contents of putrescine and spermidine in the cell variant but spermine content was little reduced (Table 1). Although putrescine and spermidine were not detectable in the cells after 2 days' putrescine starvation, cellular replication and DNA synthesis were not strongly affected until after 3–4 days (Figs 1, 2). This is in accordance with earlier results demonstrating that difluoromethylornithine, which inhibits the activity of ornithine decarboxylase⁷ and synthesis of putrescine, causes rapid depletion of polyamines in various animal cells but does not markedly inhibit cellular replication until after the second generation^{8,9}.

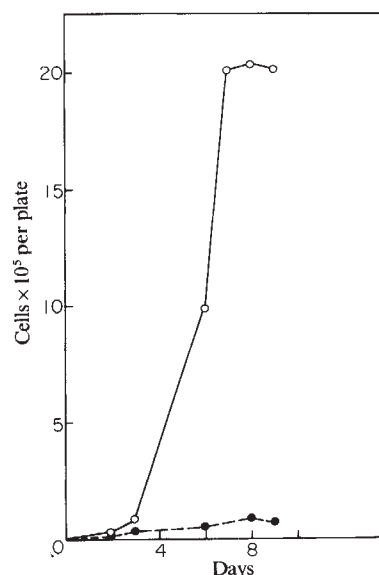


Fig. 1 Putrescine requirement for replication of the polyamine auxotroph. 5,000 cells were plated on plastic Petri dishes, diameter 30 mm, coated with pig skin collagen in serum-free MEM-F12 medium, without putrescine and supplemented with 0.1% bovine serum albumin. After 6 h incubation, putrescine (5×10^{-7} M) was added to half the cultures and the cells were detached with trypsin at different time intervals for counting in an electric cell counter. The medium was changed after 3 and 7 days. Each point is a mean of two determinations. Putrescine added (○); not added (●).

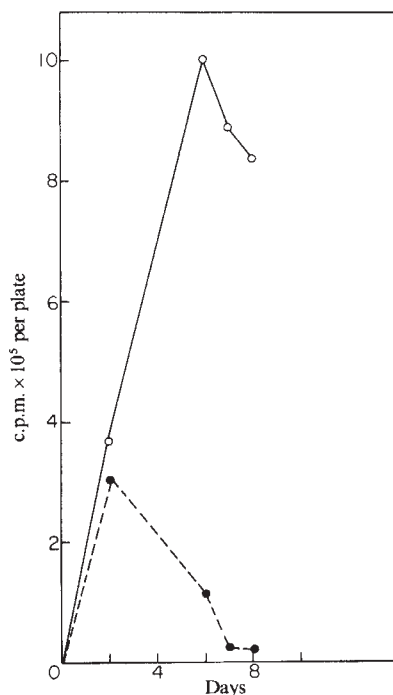


Fig. 2 DNA synthesis in the polyamine auxotroph in the absence (●) and presence (○) of putrescine. Cells were cultured as in Fig. 1 legend. To assay DNA synthesis, cells were incubated with ^3H -thymidine ($1 \mu\text{Ci ml}^{-1}$) for 24 h starting at the indicated time points and the radioactivity in the acid-precipitable fraction was determined as described earlier¹⁸. Each point is a mean of two determinations.

Although the polyamine-auxotrophic cells cultured in putrescine-free medium did not show any morphological changes under the phase-contrast microscope, indirect immunofluorescence microscopy (IIF) revealed that in more than 90% of the cells, the bundles of actin filaments were almost totally absent, only a weak diffuse fluorescence being discernible (Fig. 3b). On the other hand, the respective controls maintained in putrescine-containing medium displayed typical organization of actin, microfilaments being present in nearly all cells (Fig. 3a). The lack of putrescine also prevented the assembly of microtubules. More than 90% of cells cultured in putrescine-free medium showed only a diffuse staining for microtubules in IIF (Fig. 3d), whereas a normal cytoplasmic microtubular complex was visible in nearly all control cells grown in the presence of putrescine (Fig. 3c). In contrast, the vimentin type of intermediate filaments were not affected by putrescine limitation (Fig. 3e, f). Because the intermediate filaments form the main

Table 1 Polyamine levels in the polyamine auxotroph after putrescine starvation

Culture conditions	Duration of incubation (days)	Putrescine	Spermidine (nmol per 10^6 cells)	Spermine
+Putrescine	2	0.5	3.9	2.0
-Putrescine	2	<0.01	<0.01	1.8
+Putrescine	4	0.2	3.8	3.0
-Putrescine	4	<0.01	<0.01	1.8

2×10^6 cells were plated on 30-mm Petri dishes in the absence or presence of putrescine (5×10^{-7} M). After 2 and 4 days the cultures were washed three times with Hanks' salt solution and the cells detached by scraping, centrifuged and extracted with 0.2 M perchloric acid. The polyamine levels were determined by the dansylation method¹⁹ as described earlier²⁰.

scaffolding of the cell¹⁰, their presence explains the seemingly normal morphology of the cells starved of putrescine. Both cells deprived of putrescine for 6 days and control cells were viable, as demonstrated by the Trypan blue exclusion test. In the absence of putrescine, an increased number of binucleate cells was also usually observed (Fig. 3).

A role for polyamines in the polymerization of actin filaments has been suggested by Sunkara *et al.*¹¹, who showed that treatment of human fibroblasts with an inhibitor of *S*-adenosylmethionine decarboxylase, methylglyoxal bis(guanyldihydrazone)¹², which prevents the synthesis of spermidine and spermine, inhibited the formation of actin bundles. However, this inhibitor may be toxic to cells^{8,13}, affecting not only polyamine synthesis but also mitochondrial function¹⁴. It is therefore difficult to decide whether disruption of the actomyosin system was due to a polyamine depletion or to side effects of the drug. In the present study, no drugs were used. Omission of putrescine from the growth medium alone was sufficient to prevent polyamine accumulation, resulting subsequently in disorganization of actin bundles and microtubules. It is not a question of general disorganization of the cellular architecture as intermediate filaments, which are very sensitive to proteases¹⁵, showed normal patterns. It may be relevant that muscle actin polymerizes *in vitro* on addition of polyamines¹⁶; but it is premature to conclude that, in living cells, polyamines are directly involved in the polymerization process.

Whereas the polyamine-auxotrophic *E. coli* mutant, when depleted of polyamines, indefinitely maintained replication, albeit slow⁴, the present CHO variants ceased to replicate in polyamine-free medium. One important difference between bacteria and animal cells is that the latter possess an intricate three-dimensional filamentous cytoskeleton which is absent in bacteria¹⁷. Depletion of polyamines in animal cells may become fatal because the integrity of the microfilaments and micro-

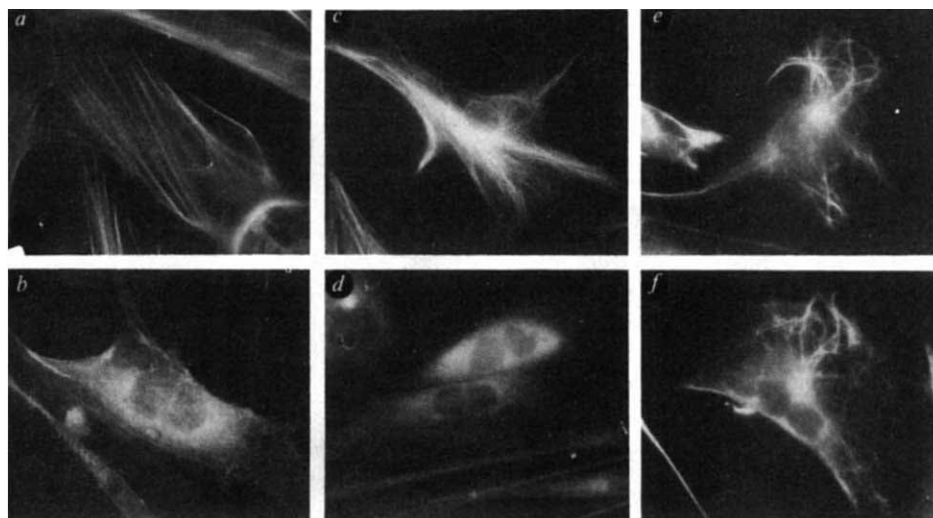


Fig. 3 Distribution of actin (a, b), tubulin (c, d) and vimentin (e, f) in control (a, c, e) and polyamine-deficient variant (b, d, f) CHO cells. For IIF the cells were grown on small glass coverslips and fixed in methanol at -20°C . Affinity-purified antibodies against actin²¹, tubulin^{21,22} and vimentin²³ were used in IIF. Anti-actin and -tubulin antibodies were kindly provided by R. A. Badley (Unilever). Typical bundles of actin-containing microfilaments (a) and cytoplasmic fibrillar patterns of microtubules (c) and vimentin filaments (e) were seen during IIF of control cells. However, cells depleted of putrescine for 6 days showed only diffuse fluorescence for actin (b) and tubulin (d), but showed typical fibrillar distribution of vimentin filaments (f).

tubules depends on polyamines and this fibrillar network is vital for cellular organization and cell division.

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A corticosteroid binding protein and endogenous ligand in *C. albicans* indicating a possible steroid-receptor system

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The evolution of endocrine systems has been intensively studied (for review see ref. 1). Recent findings indicate the existence of vertebrate-like hormones in primitive species including fungi and unicellular eukaryotes^{2–4}. Although proteins that bind vertebrate steroids have been described in at least one lower organism⁵, the presence of recognition molecules analogous to hormone receptors in higher organisms has not previously been demonstrated. We have investigated *Candida albicans* and now report the existence of a receptor-like protein macromolecule in the cytosol of this simple yeast which binds vertebrate corticosteroids with high affinity, selectivity and stereospecificity. In addition, a lipid-extractable material present in the yeast cells and released into the chemically defined growth medium reversibly competes for ³H-corticosterone binding sites in yeast cytosol and may represent an endogenous ligand. Of interest is the finding that the yeast extract also competes for mammalian glucocorticoid receptors. The data suggest that the evolutionary origin of hormonal steroid systems may go back at least as far as simple unicellular eukaryotes. Furthermore, the demonstration of interactions between mammalian steroids and the yeast binder and between the yeast ligand and mammalian receptors suggests the possibility of important clinical consequences.

Most experiments used a clinical isolate of *C. albicans*, although well characterized strains have also been examined and will be reported elsewhere (D.S.L., D. Stevens and D.F., in preparation). *C. albicans* were grown at 37 °C on agar plates or as a suspension in Dulbecco's minimum essential medium (Gibco). Yeast were collected after 24 h of growth, washed, collected by centrifugation at 1,000g, disrupted by vortexing with 250–300-μm glass beads and cytosol was prepared.

Binding experiments were routinely performed with yeast cytosol using ³H-corticosterone (Amersham; 57 Ci mmol⁻¹). Incubation for 3 h at 0 °C achieved maximal steady-state binding. Other tritiated steroids were also tested for binding activity, including oestradiol, progesterone, testosterone, diethylstilboestrol, dexamethasone and triamcinolone acetonide (all obtained from Amersham) and R5020 (Promegestone, NEN). After incubation of cytosol with the ³H-labelled probes, protein-bound steroid was separated from free steroid by chromatography over 4-ml minicolumns of G-50 fine Sephadex (Pharmacia) as previously described⁶. All studies were corrected for nonspecific binding (always <10%) by examining parallel samples incubated in the presence of a 250-fold excess of the appropriate radioinert steroid. An ethanolic extract of *C. albicans* was compared with a series of radioinert steroids for its ability to compete with ³H-dexamethasone for rat thymic glucocorticoid receptors and with ³H-corticosterone for human serum corticosteroid-binding globulin (CBG) as previously described⁷.

Preliminary experiments using various mammalian ³H-labelled steroids only revealed specific binding to *C. albicans* cytosol with ³H-corticosterone and ³H-progesterone. Radioinert corticosterone competed for the ³H-progesterone sites and radioinert progesterone competed for the ³H-corticosterone sites, suggesting that both probes were labelling the same site. Because rather higher levels of binding were found with ³H-corticosterone, we used the vertebrate glucocorticoid as our ³H-labelled probe to determine the characteristics of the yeast cytosol binder. Figure 1a illustrates a binding study with ³H-corticosterone and Fig 1b shows a Scatchard plot of the data⁸. The results indicate a single class of low-capacity, high-affinity binding sites. In five such experiments the apparent equilibrium dissociation constant was 7.2 ± 0.5 nM and the binding capacity was 732 ± 73 (±s.e.m.) fmol per mg cytosol protein.

Further studies to characterize the nature of the yeast ³H-corticosterone binder revealed that it was stable at 37 °C but virtually destroyed at 56 °C. Treatment of the cytosol with 0.1 mg ml⁻¹ trypsin for 30 min at 37 °C destroyed the binding, as did the addition of 6 mM N-ethylmaleimide. However, treatment with RNase, DNase, neuraminidase or phospholipase A₂ had little or no effect on binding. These results indicate that the binder is probably a protein with free sulphhydryl groups

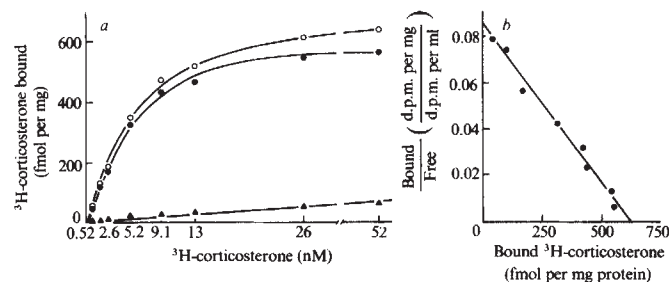


Fig. 1 a, Binding of ³H-corticosterone to *C. albicans* cytosol. After disruption of yeast, cytosol was prepared by centrifugation at 204,000g for 30 min and incubated with increasing concentrations of ³H-corticosterone for 3 h at 0 °C. Bound steroid was separated from free steroid by chromatography on Sephadex G-50 minicolumns. b, Data for specifically bound ³H-corticosterone from a analysed by the method of Scatchard. $K_d = 7.5$ nM, $N_{max} = 651$ fmol per mg protein. ○, Total; ●, specific; ▲, nonspecific.

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required for binding. The properties of the binder are described in detail elsewhere (D.S.L. and D. F., in preparation) but it is interesting that the ^3H -corticosterone-binder complex sediments at $\sim 4.0\text{S}$ in hypotonic sucrose density gradients and has an approximate molecular weight of 60,000 estimated from chromatography on exclusion gels.

The steroid specificity of the *C. albicans* ^3H -corticosterone binding protein was explored in a series of competition experiments and the data are presented in Fig. 2. Radioinert corticosterone and progesterone were potent displacers of the ^3H -corticosterone binding, 11β -cortisol and prednisolone were moderate competitors, while the potent synthetic glucocorticoid, dexamethasone, failed to bind. R-5020, a synthetic progestin with high affinity for mammalian progesterone receptors⁹, had 2% of the activity of progesterone. The sex steroids oestradiol and testosterone, the mineralocorticoid aldosterone and 11α -cortisol, an inactive (in mammals) stereoisomer of 11β -cortisol, were all devoid of activity. Ergosterol, the major sterol synthesized by *C. albicans*, displaced only 3% of the bound ^3H -corticosterone at 100-fold molar excess (data not shown).

We next investigated whether a native endogenous ligand existed which could specifically occupy this yeast binding site. A crude ethanolic extract of *C. albicans* yielded a material with the ability to compete with ^3H -corticosterone for the fungal binding site (Fig. 3a). Lineweaver-Burk analysis indicated that the displacement was competitive in nature. Furthermore, as shown in Fig. 3b, c, this extract had the ability competitively to displace ^3H -dexamethasone from classic glucocorticoid receptors in rat thymus cytosol as well as ^3H -corticosterone from human plasma CBG. However, the yeast extract was considerably more potent in inhibiting binding to the fungal protein. Although we have not identified the active factor(s) in this ethanolic extract of *C. albicans*, we believe the binding activity to be due to a native endogenous ligand, perhaps a fungal sterol. Similar binding activity was detected in methylene chloride extracts of growth media from *C. albicans*.

The studies described here demonstrate the existence of a protein binder in the cytosol of *C. albicans* which has high affinity and considerable specificity for vertebrate steroids. The binder exhibits properties consistent with a receptor molecule. It is stereospecific, extremely selective and has a high affinity for ^3H -corticosterone ($\sim 7\text{ nM}$) which is equivalent to that of mammalian glucocorticoid receptors⁷. The number of binding sites (730 fmol per mg protein) is in the range of the receptor binding capacity reported for many mammalian glucocorticoid systems⁷. Lipid extraction of *C. albicans* or its culture medium yielded a material which reversibly competes for yeast cytosol

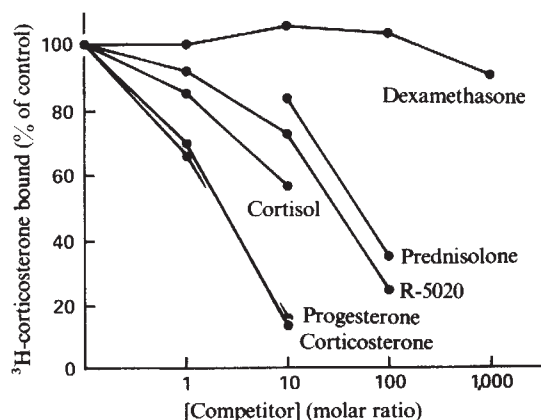


Fig. 2 Specificity of the *C. albicans* cytosol binder. Cytosol was incubated with 13 nM ^3H -corticosterone $\pm$ competitor steroids at the indicated molar ratios for 3 h at 0°C . Control (100%) binding in the absence of competitors was 486 ± 45 (s.e.m., $n = 5$) fmol per mg cytosol protein.

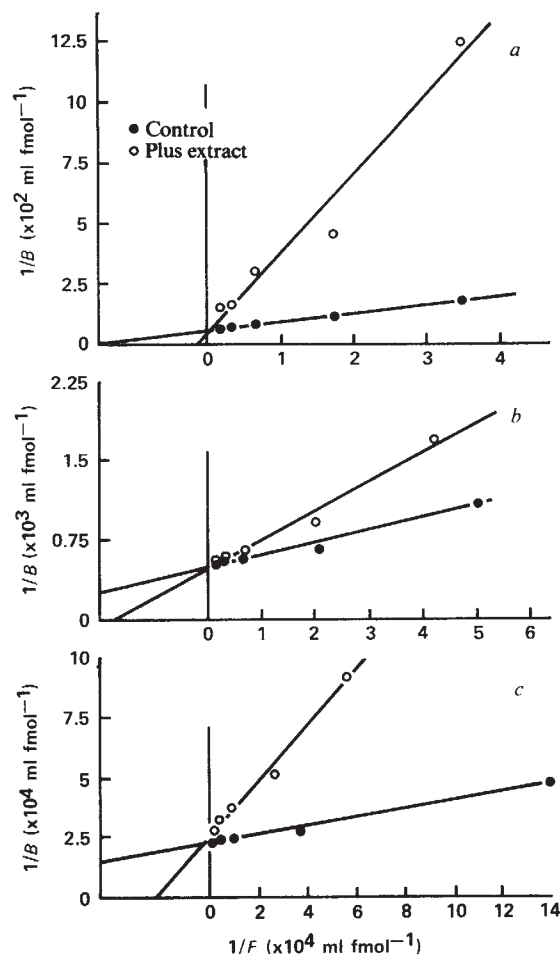


Fig. 3 Lineweaver-Burk analysis of the ethanol-extractable *C. albicans* ligand indicates competitive binding to three steroid binding proteins. *C. albicans* ($\sim 5 \times 10^9$ organisms) were extracted for 24 h with redistilled ethanol to a final extract volume of 10 ml. Aliquots of yeast extract were dried down to remove ethanol and then examined for their ability to compete with vertebrate steroids for specific binding sites. *a*, *C. albicans* cytosol incubated with 13 nM ^3H -corticosterone $\pm 50\text{ }\mu\text{l}$ yeast extract. *b*, Adrenalectomized rat thymus cytosol incubated with 13 nM ^3H -dexamethasone $\pm 100\text{ }\mu\text{l}$ yeast extract. *c*, Human serum incubated with 13 nM ^3H -corticosterone $\pm 200\text{ }\mu\text{l}$ yeast extract.

binding sites as well as mammalian receptor binding sites. We hypothesize that this substance represents the endogenous ligand for the cytosol binder.

These results suggest that *C. albicans* possesses both components of a hormone-receptor system—a ligand and a binding protein. The evolution of the mammalian steroid hormone-receptor system and what seems to be an analogous system in simple unicellular eukaryotic yeast suggests that both may be derived from a common ancestor.

The ability of mammalian steroids to interact with the *C. albicans* binder and the ability of the yeast lipid extractable factor to compete for mammalian binding sites raise the possibility of important interactions between infecting organism and host. *C. albicans* infects man and has a special predilection for patients receiving glucocorticoid therapy. We speculate that the hormonal milieu of the host may act via the *C. albicans* receptor to alter the behaviour of the infecting yeast. Conversely, the endogenous ligand from *C. albicans* may modify host cellular function by interacting with steroid receptors in various target organs. We believe these new considerations warrant intense investigation.

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Biochemical identification of viruses causing the 1981 outbreaks of foot and mouth disease in the UK

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The viruses causing the recent outbreaks of foot and mouth disease in the United Kingdom (Jersey and the Isle of Wight) and France (Brittany and Normandy) have been shown by analysis of the virus-induced proteins and the viral RNAs to be closely related to each other, and to another virus of serotype O, Lausanne 1965 (Switzerland), which is used in the preparation of commercial vaccines. Comparative studies described here and elsewhere^{1,2} indicate that foot and mouth disease virus (FMDV) is extremely unlikely to survive unaltered in the field for 16 yr. Thus the outbreaks must have been caused by the re-introduction into the field of the Lausanne strain, possibly by escape from a laboratory, by the use of a vaccine contaminated with the virus or by an incompletely inactivated foot and mouth disease vaccine.

The vaccination policy adopted on the European mainland has greatly reduced the incidence of foot and mouth disease and has also benefited the United Kingdom, where vaccination is not used. Indeed, the recent outbreaks in Jersey and the Isle of Wight (March 1981) were the first in the United Kingdom since 1974. They coincided with an outbreak in Normandy and followed closely an outbreak in Brittany. Weather conditions at the time were suitable for the virus to be spread by the wind from France to the United Kingdom³.

In all four cases, the causative viruses were shown by the World Reference Laboratory of this institute to be closely related to members of serotype O, subtype 1. To characterize these isolates more precisely, we have used two independent biochemical methods: electrofocusing of virus-induced polypeptides from infected cells and fingerprinting of ribonuclease T₁ hydrolysates of the virus RNA. By these means we have found molecular evidence that the four outbreaks were caused by the same strain of virus, and, more importantly, that this strain is indistinguishable from the Lausanne strain of virus first isolated in 1965, but clearly different from many other viruses of serotype O.

Electrofocusing separates proteins according to their charge. The technique has been little used for comparing viruses although it provides a sensitive and rapid method for distinguishing closely related strains. Using electrofocusing, electrophoretic mutations affecting virus proteins have been detected in viruses collected at different times¹ and in conditional lethal mutants isolated in the laboratory^{4,5}. At least eight polypeptides, accounting for 75% of the coding capacity of the

genome, can be resolved in a simple one-dimensional analysis.

Figure 1 illustrates the application of the method to 16 different isolates of FMDV, serotype O. To focus all the polypeptides it is necessary to use both electrophoretic polarities; hence Figs 1 and 3 are each composed of two independent analyses, joined so that redundant information is omitted. To reduce the background of cellular protein, virus-induced polypeptides were separated from cell extracts by precipitating them with convalescent serum⁶. The identification of the polypeptides on the gels was based on two-dimensional analyses (not shown) performed as described previously⁵ for three strains of serotype O, one of which (O-BFS 1860, Fig. 1k) was a virus isolated during the extensive outbreak of disease in the United Kingdom in 1967-68. The polypeptides of all other isolates were identified by analogy with these reference strains.

Examination of about 60 different viruses of serotype O showed that the net charge of two of the eight polypeptides (VP4 and P34) is highly conserved, as shown in Fig. 1 for the 16 isolates. Furthermore, VP2 and P56a varied in the same manner as in their precursor polypeptides, P38 and P72 respectively. Comparisons of viruses were based, therefore, on the four polypeptides VP1, VP2, VP3 and P56a. Differences in the charge of FMDV proteins are believed to manifest differences in amino acid sequence rather than degrees of phosphorylation⁵. Figure 1a-g shows the patterns of polypeptides produced by

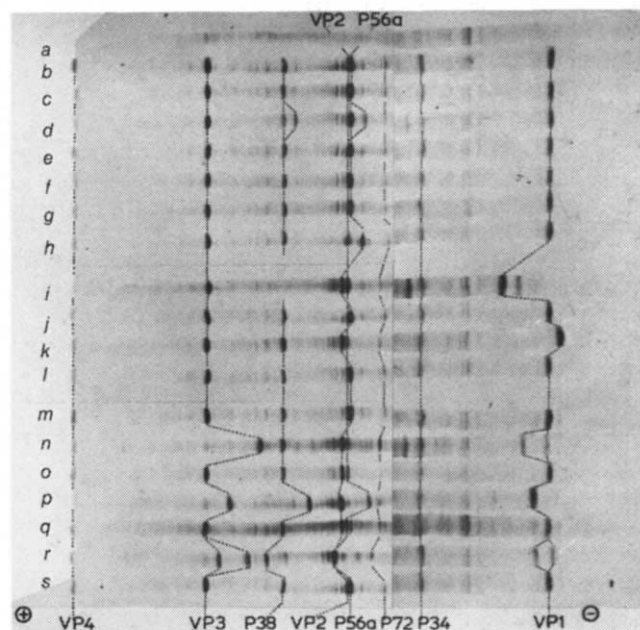


Fig. 1 Electrofocusing FMDV-specific polypeptides from infected cells: comparison of isolates from the 1981 outbreak in the United Kingdom and France with other type O isolates. Virus samples were supplied as epithelium from infected animals or such material passaged once in primary bovine thyroid cells. After passaging the virus two to four times in BHK 21 cells, cytoplasmic extracts were prepared from virus-infected BHK cells labelled with ³⁵S-methionine as described previously⁵. Virus-specific proteins were precipitated using convalescent guinea pig antiserum to FMDV strain O Pacheco⁶. Washed immune precipitates were resuspended in 0.1 ml of 0.01 M Tris-HCl pH 7.4, 1 mM EDTA, 1% (v/v) NP40 and 0.1 mg ml⁻¹ pancreatic ribonuclease, and incubated for 15 min at 37 °C. Mercapto-ethanol (3.5 µl) and urea (100 mg) were added and the solution incubated for a further 15 min at 37 °C. Up to 20 µl of each radiolabelled polypeptide solution were electrofocused as described previously. The figure is composed from parts of two sets of electrofocusing gels, run with opposite polarity and joined so that redundant information at the origins of the gels is omitted. On the right, electrofocusing was as for method (i) in ref. 4, except that 2% NP40 was included in the gels. The gels on the left were prepared using a mixture of LKB ampholines: pH 2.5-4, pH 4-6, pH 5-7 and pH 3.5-10 in the ratio 1:1.5:1:1.5. Electrofocusing was for 1 h at 200 V, 4 h at 400 V and 2 h at 600 V. a, Uninfected; b, Brittany 1981; c, Normandy 1981; d, Jersey 1981; e, Isle of Wight i 1981; f, Isle of Wight ii 1981; g, Isle of Wight iii 1981; h, Isle of Wight iv 1981; i, Israel 1963; j, Lausanne 1965; k, BFS 1860 1967; l, f + j; m, France 1979; n, India 1979; o, Malaysia 1980; p, Thailand 1980; q, Saudi Arabia 1981; r, Austria 1981; s, f + m.

viruses isolated from the recent outbreaks which started in Brittany (Fig. 1b). It can be seen that very similar viruses subsequently appeared in Normandy (Fig. 1c), Jersey (d) and the Isle of Wight (e-h). Indeed, the only change in polypeptide charge was in the P56a of one of the Isle of Wight samples (Fig. 1h). An additional VP2 band was found in the Jersey virus, presumably indicating that it was a mixture of viruses. It may be significant that difficulty was encountered in recovering virus from these two samples.

The other viruses shown in Fig. 1i-r were studied as potential sources of the outbreak. Figure 1i-k shows the patterns produced by three viruses belonging to subtype 1, isolated many years ago, which have subsequently been used for the production of vaccines, and Fig. 1m-r shows some recent field isolates of serotype O. There is considerable variation among these

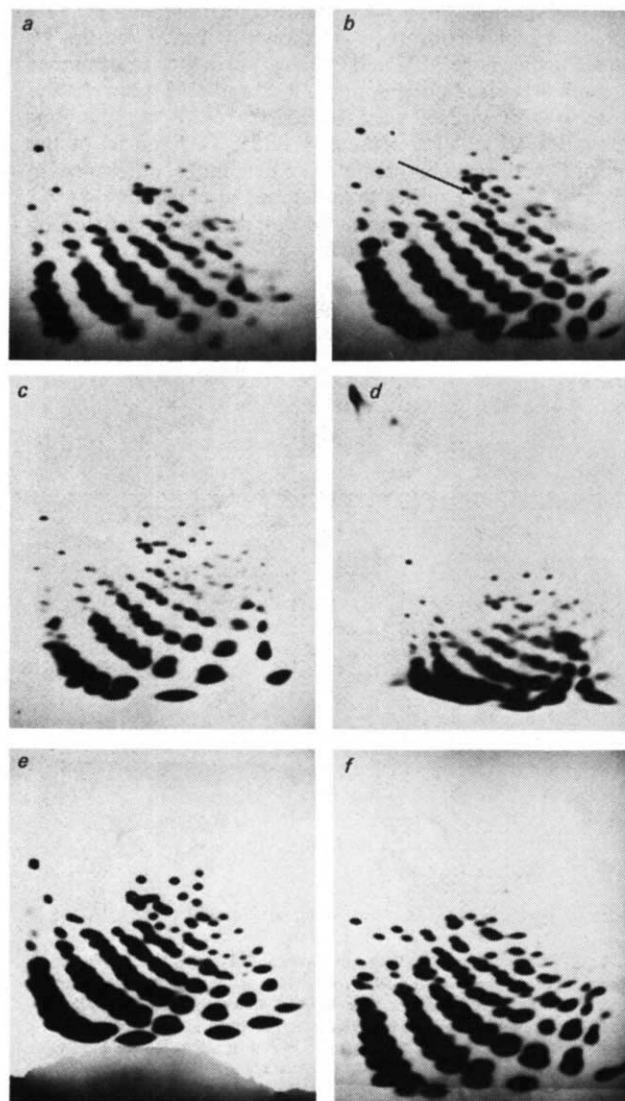


Fig. 2 RNase T₁ fingerprints of virus RNAs prepared from RNA extracted from purified ³²P-labelled virus particles. The virus isolates were grown in BHK 21 cells in Earle's medium in which the phosphate had been replaced with Tris buffer pH 7.6, and contained ~100 µCi ³²PO₄ ml⁻¹. The virus particles were purified essentially as described previously⁷ and the RNA extracted from the sucrose gradient fractions with phenol in the presence of 0.1% SDS, 0.1 M Na acetate pH 5.0. The aqueous layer containing 50 µg tRNA was precipitated with 2 vol ethanol at -20 °C overnight, the RNA collected by centrifugation and hydrolysed with RNase T₁. The mixture of oligonucleotides was analysed by two-dimensional electrophoresis essentially as described previously⁸. a, Brittany; b, Jersey; c, Brittany and Lausanne; d, Lausanne; e, Isle of Wight; f, Austria.

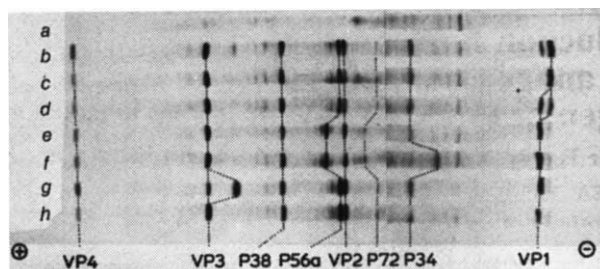


Fig. 3 A comparative electrofocusing study of polypeptides induced by FMDVs isolated at different times during the UK outbreak of 1967-68. Isolates are identified by World Reference Laboratory ref. numbers. Methods were as for Fig. 1. a, Uninfected; b, BFS 1848 Nov 1967; c, BFS 1860 Nov 1967; d, BFS 1891 Nov 1967; e, BFS 1999 Jan 1968; f, BFS 59 Mar 1968; g, BFS 86 May 1968; h, BFS 88 May 1968.

viruses, each of the variable polypeptides having several different possible isoelectric points. The UK 1981 isolates showed no similarity to the Austrian isolate (Fig. 1r) which is the only other outbreak of serotype O to occur in Europe this year. However, the most striking result is that two viruses, Lausanne 1965 (Fig. 1j) and a virus isolated in Normandy in 1979 (Fig. 1m), produced identical polypeptide patterns to the 1981 isolates in France and the United Kingdom. This identity was confirmed by electrofocusing mixtures of the polypeptides (Fig. 1l, s). These results suggest that the causative viruses in the outbreak in France in 1979 and in the United Kingdom and France in 1981 were closely related to the Lausanne strain which is used in the preparation of some commercial vaccines.

Two-dimensional fingerprints of ribonuclease T₁ digests of the isolated RNAs provided more detailed evidence that the Brittany, Normandy and Isle of Wight viruses were indistinguishable from the Lausanne virus (Fig. 2). The identity of the fingerprints of the Brittany and Lausanne viruses was confirmed by analysing a mixture of the ribonuclease T₁ digests (Fig. 2c); the mixture gave the same number of spots as the individual RNAs. The Jersey virus gave a fingerprint identical to those from the other viruses except for one additional spot (arrowed in Fig. 2b). Several other viruses of serotype O, including Austria 1981 (Fig. 2f), India 1979 and Thailand 1980, gave different fingerprints (not shown).

The finding that the 1981 UK isolates and a virus first isolated 16 yr ago were identical was surprising because continuous passage of foot and mouth disease in animals over such a long period would be expected to alter considerably its polypeptide and oligonucleotide patterns¹. Further evidence of frequent mutational change was found by electrofocusing polypeptides induced by a selection of seven isolates from the 1967-68 outbreak in England. Only two of the seven isolates gave identical patterns (Fig. 3). We therefore consider it unlikely that the Lausanne virus would have survived unaltered in field conditions for 16 yr. More likely explanations for its recurrence in the field this year are (1) its escape from a laboratory, (2) the use of a vaccine contaminated with the virus, or (3) the use of an inactivated foot and mouth disease vaccine.

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Production in *B. subtilis* of hepatitis B core antigen and of major antigen of foot and mouth disease virus

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Although *Escherichia coli* has generally been used as the host for expressing cloned genes, there may be advantages in using other bacteria, such as *Bacillus subtilis*, for making viral or eukaryotic polypeptides. *B. subtilis* is a non-pathogenic Gram-positive bacterium which, unlike *E. coli*, does not produce endotoxins and excretes several extracellular proteins in large amounts. Strains of *Bacillus* are widely used commercially for producing enzymes and antibiotics, and *B. subtilis* (natto) is used in Japan as a source of food for human consumption. Existing methods for large-scale growth of *B. subtilis* and for isolating proteins from such cultures may be useful for making viral or eukaryotic polypeptides from *Bacillus* strains containing cloned DNA. We describe here how proteins cross-reacting with antibodies against either the core antigen of hepatitis B virus (HBV) or the major antigen (VP1) of foot and mouth disease virus (FMDV) can be made in *B. subtilis* when the appropriate viral gene is inserted into a suitable plasmid vector.

To express genes coding for viral proteins in *B. subtilis*, viral DNA was inserted into a plasmid gene for erythromycin resistance. Plasmid pBD9 (refs 1,2) comprises genes coding for resistance to kanamycin and to erythromycin. The gene for erythromycin resistance encodes a protein of molecular weight (MW) 29,000, whose synthesis in *B. subtilis* is induced by sub-inhibitory concentrations of erythromycin³. The sequence of the erythromycin resistance gene has been determined⁴.

Plasmid pPLVP1 is a derivative of pBR322 in which a DNA fragment coding for all except eight amino acids at the NH₂ terminus of VP1 is joined to DNA coding for 99 amino acids of the replicase gene of bacteriophage MS2 (ref. 5). The plasmid has a unique *Bam*HI site at the junction of the FMDV and MS2 replicase fragments (Fig. 1).

Plasmid pPLVP1 which has been cut with *Bam*HI can be joined to pBD9 which has been cut with *Bcl*I, as both these enzymes cleave DNA to give a single-stranded sequence of GATC at the 5' end (Fig. 2). In one of the two possible orientations, pPLVP1 and pBD9 should join to form a fusion polypeptide of 370 amino acids, comprising 73 amino acids encoded by the erythromycin resistance gene, 284 amino acids of FMDV and 13 amino acids of the vector. Thus, the FMDV-derived sequences should code for all except eight amino acids at the NH₂ terminus of the VP1 protein. VP1 is composed of 213 amino acids⁶.

After digestion of pPLVP1 and pBD9 with *Bam*HI and with *Bcl*I respectively, mixtures of the two plasmids were incubated with T4 DNA ligase and transformed into *B. subtilis* strain BD170 (Fig. 1)⁷. The plasmid in one of the transformants, pEVP1, comprises pBD9 and pPLVP1 joined together such that a fusion protein should be formed in which the VP1 protein is joined to part of the protein encoded by the erythromycin resistance gene. In pEVP2, the two plasmids are joined in the opposite orientation and no such fusion protein should be formed.

To determine whether a fusion protein of the expected molecular weight (41,000) was formed in *B. subtilis*, extracts fractionated on an SDS-polyacrylamide gel were analysed by radioimmunoassay for antigens reacting with VP1-specific antibodies (Fig. 3). Bacteria were grown in the presence of a sub-inhibitory concentration of erythromycin (0.05 µg ml⁻¹) to induce the synthesis of polypeptides produced under the control of the regulatory signals of the erythromycin resistance gene³. Extracts of BD170 (pEVP1) contained an antigen of MW ~41,000 which reacted specifically with anti-VP1 antibodies

(Fig. 3). No such antigen was present in BD170 (pEVP2) or BD170 (pEVP1) not induced with erythromycin.

To effect expression of the gene for HBV core antigen, the plasmid vector pKH80 was made by joining pBD9 to pBR322 (ref. 8) as described in Fig. 1. Plasmid pKH80 confers erythromycin resistance on *E. coli*; at high concentrations (60 µg ml⁻¹ in L-agar), erythromycin partially inhibits *E. coli* unless the plasmid gene for erythromycin resistance is present. The 29,000-MW protein specified by the erythromycin resistance gene can be detected in *E. coli* minicells containing pKH80 which have been induced with erythromycin (2 µg ml⁻¹) (K.H., unpublished observation).

To insert the HBV core gene into the single *Bcl*I cleavage site of pKH80, which occurs within the gene for erythromycin resistance⁴, plasmid pH98, which comprises the HBV core gene with cleavage sites for *Bam*HI close to each end of the gene, was isolated as described in Fig. 4.

The *Bam*HI-generated fragment from pH98 can be inserted into the *Bcl*I site of pKH80 with the proper reading frame for expressing HBV core antigen maintained (Fig. 2). Ribosomes translating the mRNA of the erythromycin resistance gene should arrive in phase at a termination codon in the HBV DNA. Three base pairs (bp) after this codon is the AUG start codon for the HBV core antigen gene, from which translation should restart *de novo* from the transcript of the viral DNA sequence (Fig. 2). A discrete core antigen polypeptide, rather than a

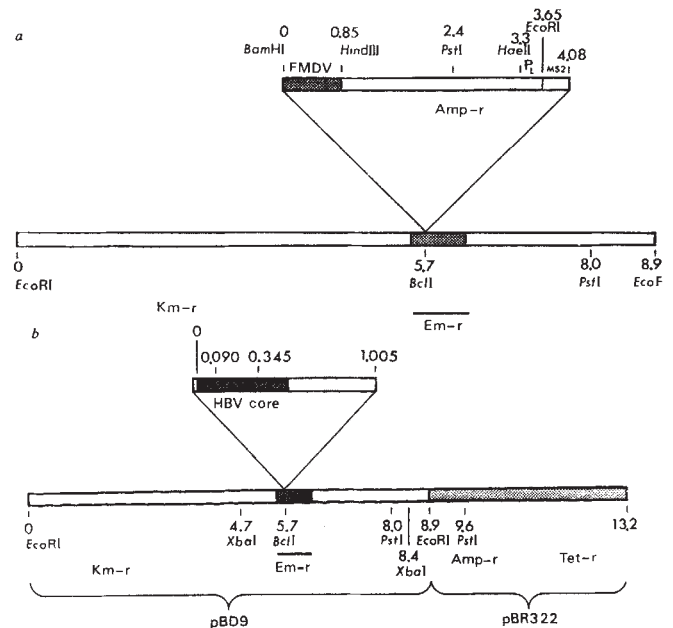


Fig. 1 a, Restriction endonuclease cleavage map of plasmid pEVP1. The lower line represents pBD9 (refs 1, 2). Km-r, approximate region coding for kanamycin resistance; Em-r, region coding for erythromycin resistance. The upper line (on a different scale) represents pPLVP1 (ref. 5). FMDV, insert-derived FMDV cDNA; P_L, AP_L control element; MS2, DNA derived from bacteriophage MS2. The numbers are kilobase coordinates. Plasmids pBD9 and pPLVP1 were digested with *Bcl*I and *Bam*HI respectively, ligated and used to transform *B. subtilis* strain BD170 (genotype, *trpC2 thr-5*) using the method of Contente and Dubnau¹². Plasmid DNA isolated from transformants resistant to kanamycin (5 µg ml⁻¹) was digested with *Eco*RI and *Pst*I and with *Hind*III and *Pst*I to determine the orientation of pPLVP1 which was inserted into the erythromycin resistance gene of pBD9. b, Restriction endonuclease cleavage map of pKH80 and derivatives containing the HBV core gene. pKH80 was made by ligating *Eco*RI-digested pBD9 to *Eco*RI-digested pBR322. In pKH81 and pKH82, a *Bam*HI-generated fragment comprising the HBV core gene was inserted at the *Bcl*I cleavage site of pKH80 in the orientation shown. In pKH83, the *Bam*HI-generated fragment was inserted in the opposite orientation. Positions of restriction endonuclease cleavage sites are those of Gryczan *et al.*² and of Horinouchi and Weisblum⁴ (for pBD9), and of Sutcliffe¹³ (for pBR322). Restriction endonucleases were obtained from New England Biolabs and used in conditions recommended by the manufacturers. The method for electrophoretic separation of DNA fragments and the conditions for ligation of DNA fragments are described elsewhere¹⁴. The upper line representing the HBV core gene is drawn to a different scale. Coordinates 0.090 and 0.345 indicate the positions of *Xba*I sites which were used to determine the orientation of HBV core inserts in the erythromycin resistance gene.

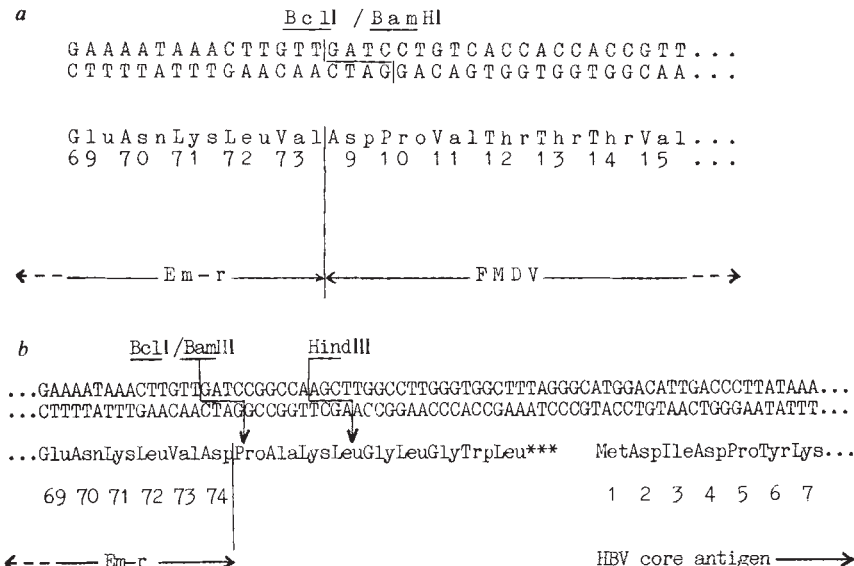


Fig. 2 *a*, Sequence at the junction of FMDV DNA inserted into *BclI* site of the erythromycin resistance gene. The predicted sequence is based on the published sequence for the erythromycin resistance gene⁶ and the gene for VP1⁶. *b*, Sequence at the junction of the HBV core gene inserted into the *BclI* site in the erythromycin gene. *** Indicates a termination codon. The sequence to the right of the erythromycin resistance gene was determined in plasmid pH98 (Fig. 4) by the method of Maxam and Gilbert¹⁵. The sequence of the erythromycin resistance gene is that of Horinouchi and Weisblum⁴.

fusion protein, should therefore be formed. Expression of the HBV core antigen gene from this type of construction was observed in *E. coli* containing the original plasmids expressing the gene⁹.

The 1,037 bp fragment produced by *BamHI* digestion of pH98 was ligated to *BclI*-digested pKH80 and recombinant plasmids, which did not confer erythromycin resistance, were selected by transformation of *E. coli* K-12 strain HB101. In plasmids pKH81 and pKH82, the HBV core gene is inserted so that it is read in the same direction as the erythromycin gene; in pKH83, the insert is in the other orientation (Fig. 1).

B. subtilis strains MI119 (ref. 10), which has the genotype *leuB6*, *trpC2*, *r⁻m⁻*, and SL650 (obtained from P. Piggot), of

genotype *spoIIA69*, *trpC2*, *rif-2*, were transformed with these plasmids and tested for HBV core antigen production by solid-phase radioimmunoassay¹¹. SL650 produces neither the spores nor some of the proteases produced by sporulation-proficient *B. subtilis*. Colonies formed during overnight incubation on plates containing erythromycin (0.05 µg ml⁻¹) were lysed by adding 5 µl of a lysozyme solution (2 mg ml⁻¹ in water) to each colony and incubating for 30 min at 37°C. Strains MI119 and SL650, containing plasmids pKH81 and pKH82, gave positive

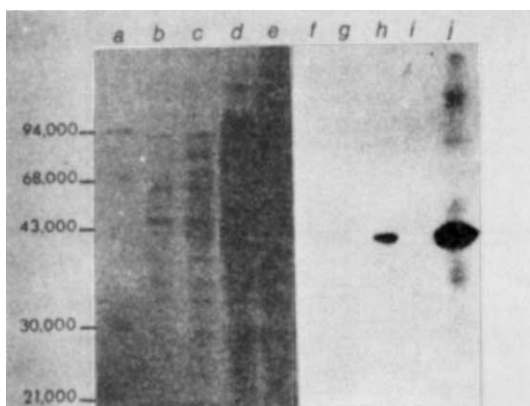


Fig. 3 *B. subtilis* BD170 containing pEVP1 or pEVP2 were grown with vigorous aeration at 37°C in 100 ml of VY broth⁷ to $A_{550}=0.2$. After addition of erythromycin (0.05 µg ml⁻¹), the cultures were grown for a further 2 h, centrifuged and resuspended in 1 ml of lysozyme buffer (25% sucrose in 30 mM Tris-HCl pH 8.0, 50 mM EDTA, 50 mM NaCl) containing lysozyme (1 mg ml⁻¹). After 30 min incubation at 37°C, the cells were lysed at 0°C by sonication. The lysate was centrifuged at 12,500 r.p.m. for 15 min at 0°C in a Sorvall SS-34 rotor. The supernatant was analysed by electrophoresis through an SDS-polyacrylamide gel (10%) and proteins in the gel transferred by a sandwich technique to two nitrocellulose filters, essentially as described by Bowen *et al.*¹⁶. To detect antigens in a filter which could bind VP1-specific antibodies, the filter was first incubated for 8 h at room temperature in a buffer comprising 2% fetal calf serum in phosphate-buffered saline to saturate protein binding sites. The filter was then incubated with 40 ml of the same buffer containing ¹²⁵I-labelled anti-VP1 IgG (6 × 10⁶ c.p.m. per µg; total of 30 × 10⁶ c.p.m. in 40 ml) overnight at room temperature. After thorough washing in the same buffer containing 0.05% NP40 (Fluka), the filter was autoradiographed. The second filter was stained for 15 min with 0.1% aniline blue black in 45% methanol, 10% acetic acid and 45% water. It was destained for 15 min with (three changes of) 50% methanol, 2% acetic acid and 48% water. Lanes *a-e*, stained nitrocellulose filter; *f-i*, autoradiograph of tracks equivalent to *a-e*. Lanes *a*, *f* protein molecular-weight markers; *b*, *g*, BD170 (pEVP2); *c*, *h*, BD170 (pEVP1); *d*, *i*, *E. coli* K12 ΔH1; *e*, *j*, *E. coli* K-12 ΔH1 (pPLVP1) (see ref. 5).

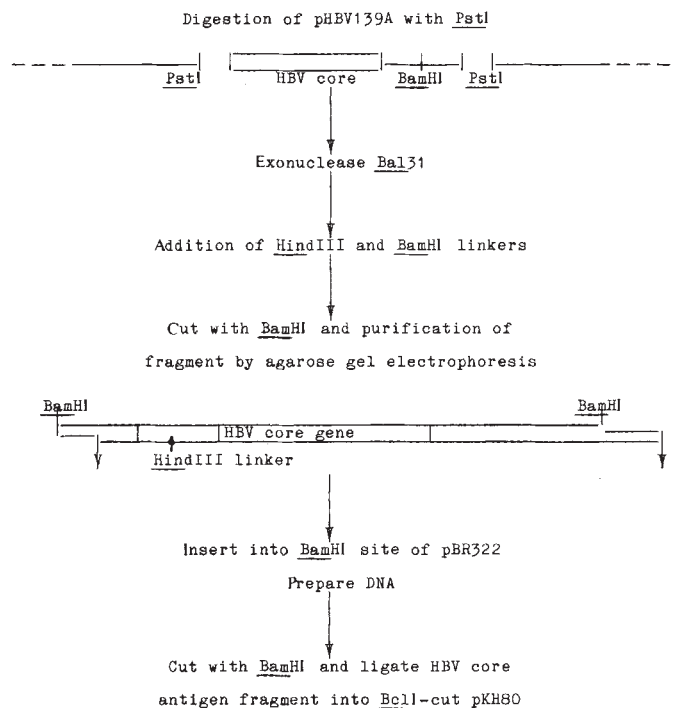


Fig. 4 Construction of *BamHI*-excisable HBV core antigen gene. *PstI*-digested pHBV139A (30 µg)⁹ was digested with 3.3 U exonuclease *Bal31* (Bethesda Research Laboratories) in 300 µl of buffer (20 mM Tris-HCl pH 8.0, 12 mM CaCl₂, 12 mM MgCl₂, 600 mM NaCl, 1 mM EDTA) for 4 min at 30°C. The reaction was stopped by phenol extraction and the DNA precipitated with ethanol. An average of ~100 bp were removed from each end of the DNA fragment. The exonuclease-treated DNA was resuspended in 20 µl of ligase buffer¹⁴ containing T4 DNA ligase and 1 µg each of *HindIII* and *BamHI* linkers (Collaborative Research). After ligation for 16 h at 15°C, the DNA was treated with *BamHI*, the fragments containing the HBV core antigen gene were purified by agarose gel electrophoresis and cloned into the *BamHI* site of pBR322. Plasmid DNA isolated from transformants which were resistant to ampicillin (40 µg ml⁻¹) and sensitive to tetracycline (15 µg ml⁻¹) were sequenced at the junction between pBR322 and the 5' end of the core antigen gene using the method of Maxam and Gilbert¹⁵.

results when tested for HBV core antigen. The same strains containing plasmid pKH83 gave negative results.

These results show that proteins encoded by animal viruses can be made in *B. subtilis*. The gene for human leukocyte interferon has also been introduced into *B. subtilis* and interferon activity detected (A. H. A. Bingham, personal communication). The product of the erythromycin resistance gene, which is not an exported protein, is made in relatively large amounts and is readily detectable in *Bacillus minicells*⁷. The VP1 protein produced by strains carrying pEVP1 comprises ~1% of cellular protein. The concentration of the HBV core protein produced by MI119(pKH81) was not determined precisely but was <0.1% of cellular protein, presumably reflecting the relative inefficiency of the stop/restart type of construction. *B. subtilis* strains synthesizing viral proteins may be useful for the large-scale production of viral antigens to be used as diagnostic reagents or as vaccines.

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Rescue of *in vitro* generated mutants of cloned cauliflower mosaic virus genome in infected plants

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Cauliflower mosaic virus (CaMV) DNA is an attractive candidate as a vector for introducing foreign DNA into plants because the DNA can be directly introduced into plants by rubbing it on to leaves^{1–4}. The CaMV genome is a circular double-stranded DNA of about 8 kilobases (kb), and has been entirely sequenced⁵. DNA isolated from the virus has a 'tangled' or 'knotted' secondary structure⁶ and contains two or three (depending on the isolate) site-specific single-strand interruptions^{5,7,8}. Cloned viral DNA, when excised from its recombinant plasmid, is infective in linear form. The infected plant can recircularize the DNA 'knot' and reintroduce the site-specific single-strand interruptions¹. For CaMV to be a useful vector, sites in the genome are required into which foreign DNA can be inserted without destroying infectivity, and ideally the inserted DNA would be expressed under viral control. Here we report the generation *in vitro* of small insertion and deletion mutations in cloned CaMV DNA. Most mutations destroy the infectivity of the viral DNA except for one, a linker insertion in the large intergenic region. Co-infection of plants with non-overlapping mutants usually leads to the production of virus particles, which seem to be produced by recombination.

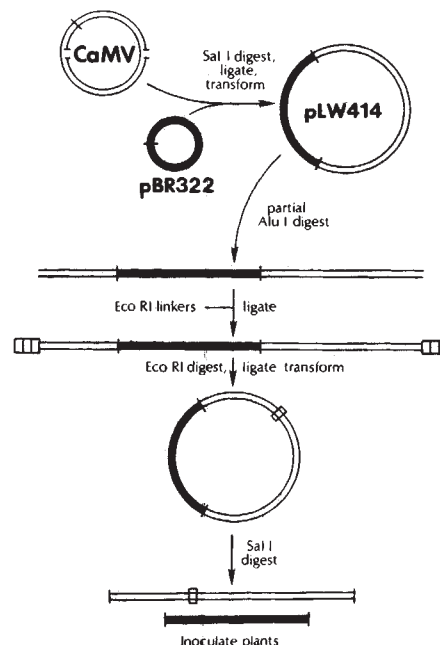


Fig. 1 Procedure for the construction of linker-insertion and deletion mutants in cloned CaMV DNA. CaMV DNA, CM4-184 isolate⁷ with two site-specific single-strand interruptions (—|—), was linearized at the single *SalI* site (—|) and inserted into the *SalI* site of pBR322 to produce pLW414. This recombinant plasmid was partially digested with *AluI* at 46 °C for 2 h in the presence of 80 µg ml⁻¹ ethidium bromide to produce a high proportion (~30%) of full-length linear molecules. Linear molecules, including full-length and some less than full-length forms, were selected by sedimentation on a 5–20% sucrose gradient. Linear molecules were methylated with *EcoRI* methylase⁹ to protect existing sites from subsequent digestion with *EcoRI*. *EcoRI* linkers, d(GGAATTCC) (Collaborative Research) in a 500 M end group excess were ligated to linear pLW414 (ref. 9). DNA with multiple attached linkers was digested with *EcoRI*, yielding plasmid molecules with linkers in monomer form and sticky ends. Such molecules were circularized with *T4* DNA ligase and used to transform *Escherichia coli* C600. Appropriate clones were selected after examining *EcoRI* digests of small-scale preparations of the cloned DNA. To inoculate plants, 20 µg of the cloned plasmid DNA were digested with *SalI*. The digest was extracted with phenol, ethanol precipitated and dissolved in 50 µl of 150 mM NaCl and 15 mM sodium citrate containing 2 mg of sterile Celite 545 (an abrasive). The inoculum was rubbed on to the leaves of three 2-week old turnip plants (*Brassica rapa* L., c.v. Just Right).

pLW414, a recombinant plasmid containing the entire CaMV (CM4-184) genome¹, was mutated *in vitro* by the introduction of deletions and linker insertions in a procedure similar to that of Heffron *et al.*⁹ (Fig. 1). Eleven modified plasmids were chosen for further study, their *EcoRI* digestion patterns being shown in Fig. 2. The parent plasmid pLW414 contains five large *EcoRI* fragments (lanes A and M): two pBR322–CaMV junctional fragments, a₂ (6.7 kb) and a₁ (1.46 kb), and three CaMV fragments, b (2.3 kb), c (1.7 kb) and d (0.46 kb). Plasmid pR49 has a new *EcoRI* site in the junctional fragment a₂, creating two new *EcoRI* fragments of 4.9 kb and 1.8 kb (lane B). Plasmid pR72 has acquired a new *EcoRI* site which splits *EcoRI* fragment c into two pieces of 1.36 and 0.28 kb (lane C). A region (0.40 kb) not bounded by linkers is deleted from *EcoRI* fragment c in pR76, yielding a smaller c fragment of 1.25 kb (lane D). In pR153 a deletion bounded by a linker eliminates fragment d and truncates fragments a₁ and b to 1.15 and 2.1 kb, respectively (lane E). Plasmid pR155 has a deletion (2.2 kb) bounded by linkers which spans the a₂–c junction, trimming fragment a₂ to 5.0 kb and fragment c to 1.20 kb (lane F). Plasmid pR173 has a large deletion bounded by linkers in which only the two shortened junctional fragments remain—fragment a₂ at 6.2 kb and fragment a₁ at 1.20 kb (lane G). A linker is inserted into fragment c of pR189, producing two new a₂ subfragments,

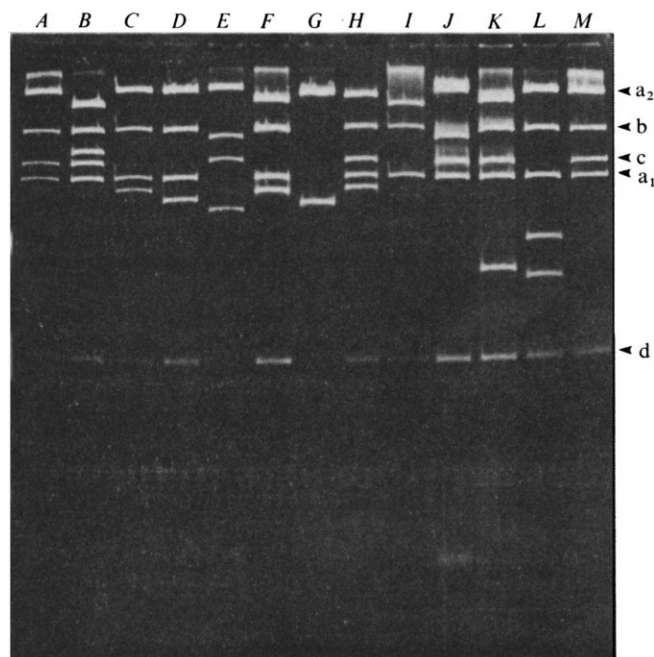


Fig. 2 *EcoRI* digest pattern of *in vitro* modified pLW414 plasmids. Plasmid DNAs (2 µg each) were digested with *EcoRI*, subjected to electrophoresis on a 5% polyacrylamide gel, stained with ethidium bromide and photographed with a UV light source. Lanes A, M, parent plasmid pLW414; lane B, pR49; lane C, pR72; lane D, pR76; lane E, pR153; lane F, pR155; lane G, pR173; lane H, pR189; lane I, pR190; lane J, pR212; lane K, pR213; lane L, pR214. Migration of *EcoRI* fragments a₂-d of pLW414 are indicated.

5.3 kb and 0.37 kb (lane H). A deletion (1.9 kb) without linkers in pR190 forms an a₂-c fusion fragment of 3.9 kb (lane I). A linker towards the end of the b fragment of pR212 splits this fragment into 2.2- and 0.12-kb subfragments (lane J). Plasmid pR213 has a deletion (0.69 kb) bounded by linkers in fragment a₂, producing subfragments of 5.2 and 0.8 kb (lane K). A linker in pR214 splits fragment c into two subfragments of 0.96 and 0.74 kb.

Maps of the modified plasmids (Fig. 3) summarize the restriction site analysis described above. These plasmids were chosen

to represent different regions of the genome and to provide plasmid pairs with closely spaced linkers (such as pR49 and pR189) or with deletions covering linker insertions (such as pR189 and pR213). The plasmid modifications cover several of the six reported potential coding regions (I-VI) described for the CaMV Cabb-S sequence⁵. For example, the linker in pR49 is in coding region V and the linker in pR72 in region I (Fig. 3).

The modified plasmids were each tested individually for their ability to infect turnip plants (*Brassica rapa* L. c.v. Just Right). The plasmids were digested with *SalI*, to excise the CaMV DNA-containing portion of the plasmid, and used to inoculate plants. Plants were scored, 4-6 weeks after inoculation, for the presence of viral symptoms: vein clearing and leaf wrinkling or stunting. Most of the plasmid modifications including linker insertions are lethal to the virus, that is, the modifications block the production of virus particles and the development of typical infection symptoms (Table 1). However, one plasmid in this series with a linker insertion, pR212, infected plants as readily as did the parent plasmid pLW414. We discuss this plasmid below.

As the mutated viral DNAs in most plasmids were non-infective on their own, we set up pairwise inoculations of all these plasmids (Table 1). In many cases, pairs of plasmids with non-overlapping modifications were able to rescue each other and infect plants, as, for example, in pR189-pR153. In no case did pairs of plasmids with overlapping modifications, such as pR189-pR213, infect plants. In many cases, plasmid pairs with closely spaced modifications did not infect plants, for example, pR72-pR214, pR49-pR189 and pR72-pR155. Although in a few other cases, pairs with closely spaced modifications, such as pR155-pR214 and pR76-pR155, were infective, only one out of three inoculated plants showed symptoms. Some pairwise inoculations with widely spaced markers did not rescue each other, as in the case of pR72-pR189 and pR76-pR213. We have repeated some of these inoculations several times with no success.

The rescue by co-infection with two mutant viral genomes probably occurs by one of two mechanisms—recombination or complementation. To determine by which mechanism rescue occurs, we analysed DNA in virus particles which result from productive infection by pairs of defective genomes. Such an analysis (Fig. 4) reveals that defective genomes are apparently rescued by recombination and that all the products of successful pairwise infections tested so far are CaMV genomes restored to their normal form. A critical test of that assumption is shown in

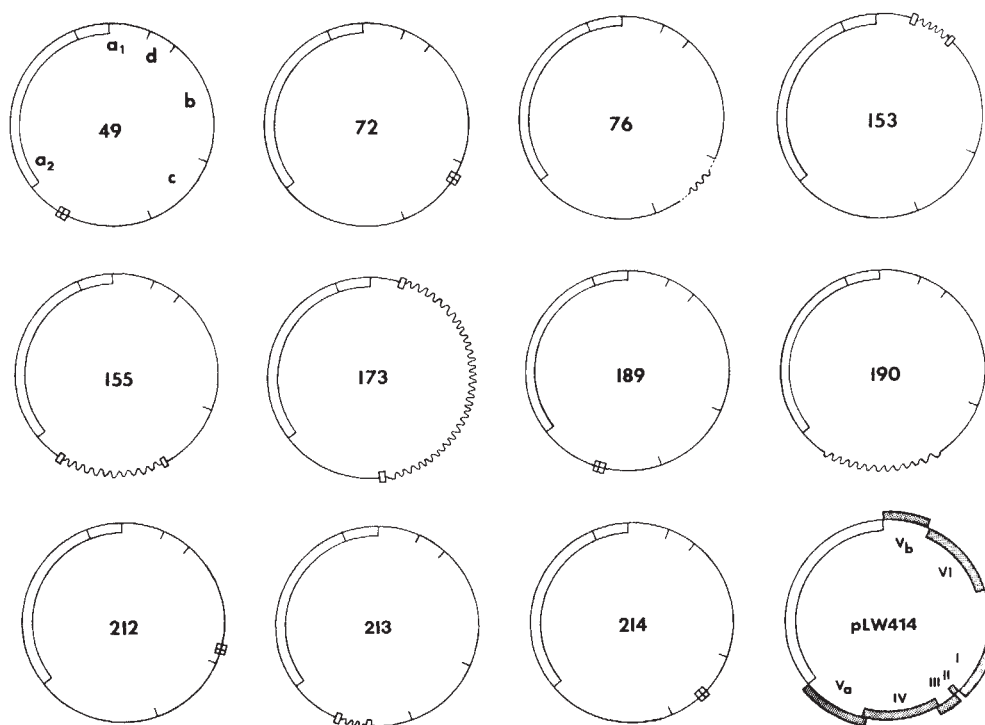
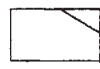


Fig. 3 Maps of linker-insertion and deletion sites in pLW414 plasmids modified *in vitro*. Data obtained from *EcoRI* digest pattern in Fig. 2 and other digests are described in text. Restriction sites (—) for the major *EcoRI* fragments a₁-d are indicated on the map of pR49 in the upper left corner. Maps are oriented so that the junction between CaMV DNA (line) and pBR322 DNA (open lozenge) in *EcoRI* fragment a₁ is at 12 o'clock. Open CaMV DNA reading frames I-VI from the sequencing data of Franck *et al.*⁵ are redrawn onto pLW414 map in the lower right-hand corner. Reading frame map is modified only to include the 421-bp deletion in reading frame II (ref. 18) found in CaMV CM4-184 isolates⁷ from which pLW414 was derived. —□—, Simple *EcoRI* linker insertion; —□—, deletion bounded by a linker; ~, simple deletion.

Table 1 Pairwise infectivity grid

	49	72	76	153	155	173	189	190	212	213	214	
	0	+	+	+	0	+	0	0	*	0	+	49
		0	0	0	0	0	0	+	*	0	0	72
			0	0	+	0	+	+	*	0	0	76
				0	0	0	+	+	*	+	ND	153
					0	0	0	0	*	0	+	155
						0	0	0	*	ND	0	173
							0	0	*	0	+	189
								0	*	0	0	190
									+	*	*	212
										0	+	213
											0	214



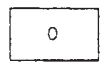
Non-overlapping lesions



Overlapping lesions



Infects



Does not infect



Infects singly, not tested pairwise

Each of three plants was inoculated with 6 μ g of each plasmid, singly or in pairwise combinations as indicated. A test was scored positive (+) if at least one out of three test plants showed symptoms. ND, not determined.

Fig. 4 (lane 5), which contains the viral DNA resulting from infection by pR76 and pR155. Neither plasmid contributes an intact *Eco*RI fragment c and yet a normal fragment c is found in the resulting viral DNA. In infections with plasmid pairs where a mixture of normal and modified *Eco*RI fragments might be expected, only normal fragments were found. In the infection with the pair pR49–pR76 (lane 3), the 1.25-kb *Eco*RI fragment from the defective pR76 genome is lost, as is the 1.8-kb subfragment from the pR49 *Eco*RI fragment a. We have also found only normal genomes resulting from infections by pairs not shown in Fig. 4, including pR76–pR189, pR152–pR213, pR153–pR189, pR189–pR214 and pR213–pR214.

Of special interest is pR212, a linker insertion in the *Eco*RI b fragment. This insertion is not a lethal mutation, and pR212 will infect plants when inoculated alone. The linker in pR212 maps in the large intergenic region between coding regions I and VI (Fig. 3), just clockwise to the single-strand interruption in the transcribed strand^{5,10,11}. Plants infected with pR212 produce virus particles that retain the linker insert in the *Eco*RI b fragment. *Eco*RI digests of viral DNA from pR212-infected plants (Fig. 4, lane 6) show that the b fragment is split into the expected 2.2- and 0.12-kb fragments (see arrows).

These findings pinpoint a possible difficulty in the use of CaMV DNA as a plant molecular vehicle when used in conjunction with helper viral DNA. The difficulty is that the foreign DNA may be expelled from the vehicle by recombinational events that take place between the vehicle and the helper. Vehicle and helper virus combinations have been quite success-

ful in the use of SV40 DNA as a vehicle when propagated as virions in animal cells (see ref. 12, for example). To infect plants directly with a CaMV vehicle and helper, it may be necessary to suppress recombination, perhaps by setting up a system to select against recombinants or by infecting with viral DNA forms that do not readily recombine. We have found that the recombination system in infected plants seems to require CaMV DNA molecules with homologous 'sticky' ends (R.M.W. and S.H.H., in preparation).

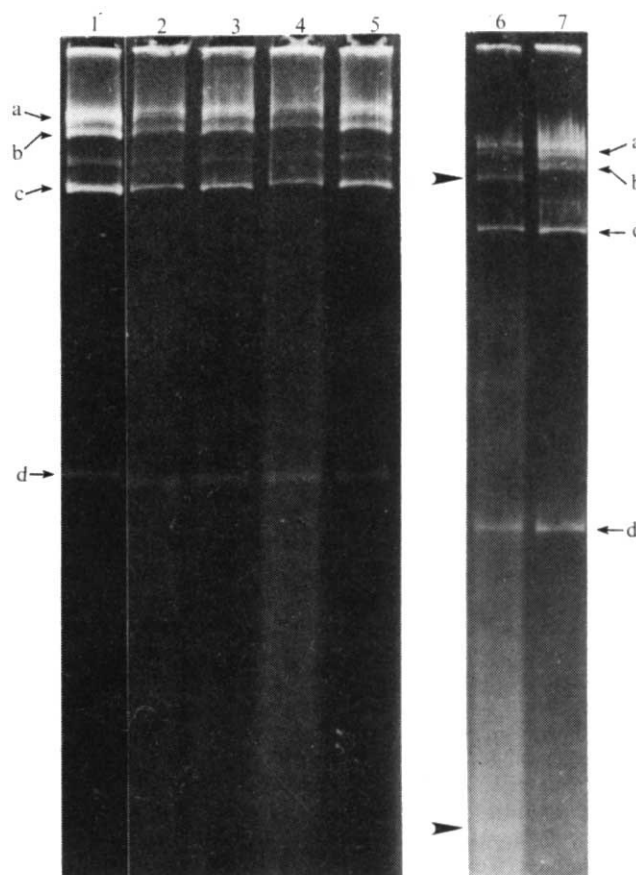


Fig. 4 *Eco*RI digest pattern of CaMV DNA obtained from small-scale viral DNA preparations of infected leaves. Viral DNA from leaves infected with pLW414 (1), pR49 and pR72 (2), pR49 and pR76 (3), pR49 and pR173 (4), pR76 and pR155 (5), pR212 (6), and pLW414 (7). Three plants were inoculated with 20 μ g of each *Sal*I-digested plasmid. Plants showed symptoms 4 weeks after infection and viral DNA was prepared from two or three leaves 1 week later using a procedure modified from Gardner and Shepherd¹⁹. The modifications include grinding the leaves to a powder in liquid nitrogen and precipitating the extracted DNA with an equal volume of ethanol. *Eco*RI fragments a–d as indicated are shown on the map of pR49 in Fig. 3. (Note that fragment a from the virus is not split into subfragments a₁ and a₂ as it is in the cloned DNA.) Minor bands between *Eco*RI-a and -b and between *Eco*RI-b and -c result from linear CaMV DNA molecules in the viral DNA preparation.

At present, it seems best to use CaMV DNA as a vehicle to insert or substitute foreign DNA at sites in the genome where insertion does not destroy infectivity. Infectivity by pR212 shows that small modifications in the intergenic region are tolerated. Gronenborn *et al.*¹³ have reported that small modifications in coding region II are also tolerated. We are interested in attempting to insert or substitute foreign DNA into the body of a structural gene that is actively expressed during CaMV infection. Coding region VI codes in the infected plant for the most abundantly accumulated messenger RNA, a 19S species¹⁴, and protein, P66 (ref. 15). P66 (molecular weight 66,000) is presumably the matrix protein of viral inclusion bodies that accumulate in the cytoplasm^{16,17}. Although these studies do not conclusively demonstrate whether P66 is essential for virus multiplication, other preliminary experiments involving site-specific insertions and deletions in this region suggest that a relatively intact region VI is required for virus production (R.M.W. and S.H.H., in preparation).

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SV40 recombinant molecules express the gene encoding p21 transforming protein of Harvey murine sarcoma virus

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Harvey murine sarcoma virus (Ha-MuSV), a replication-defective retrovirus, was originally isolated by passage of Moloney murine leukaemia virus (Mo-MuLV) in rats¹. The Ha-MuSV genome has recently been molecularly cloned and its DNA physically characterized²⁻⁴. Apart from Mo-MuLV sequences at the 5' and 3' termini of the viral genome, the other viral sequences are rat derived. One set of rat sequences is homologous to highly reiterated genes encoding 'rat 30S' RNA^{5,6}. The biological activity of cloned subgenomic fragments^{3,4,7} indicated that a second set of rat sequences (*src*) is essential for transformation and expression of the transforming protein p21, which led us to select a 1.29 kilobase pair (kbp) fragment, totally circumscribing the transforming *src* gene, for insertion into the late region of an SV40 vector. The data presented here indicate that on transmission to African green monkey kidney (AGMK) cells, this subgenomic fragment is stably expressed as a 930 base pair (bp) mRNA. This apparently unspliced mRNA encodes large quantities of a transforming protein that is indistinguishable from Ha-MuSV p21. A mRNA with a 5' end similar to that transcribed from the recombinant molecule has also been identified in HaMuSV-transformed mouse cells.

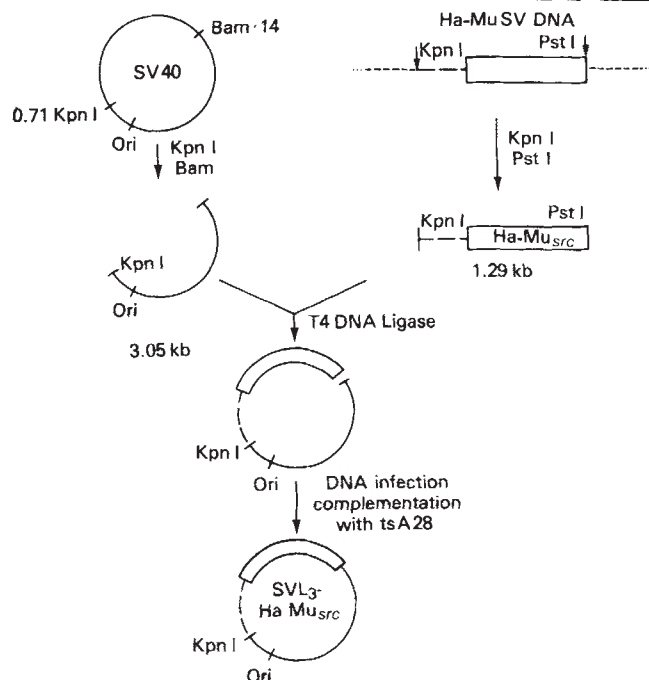


Fig. 1 Schematic representation of the construction of the SV40-Ha-MuSV recombinant. SV40 wild-type DNA was digested with *Bam*HI, treated with bacterial alkaline phosphatase (BAP) and recleaved with *Kpn*I; the larger (3 kb) fragment was isolated by agarose gel electrophoresis. In parallel, a plasmid carrying the cloned DNA fragment from Ha-MuSV was digested with *Pst*I, treated with BAP, recleaved with *Kpn*I and a 1.29 kb fragment isolated. Ligation of the two fragments was performed in conditions described previously²⁰. The ligated products were electrophoresed in an agarose gel and the heterologous dimers of ~4.3 kb were electroeluted. 20 ng of this material were used in an infectious centre assay⁸ complemented by helper tsA28. Individual plaque isolates were grown up to yield large stocks and used subsequently for infection of AGMK cells. DNA was prepared according to Hirt²⁸ and the physical map established (data not shown). A mutant with the predicted structure of SVL₃-Ha-MuSV_{src} was selected and used for further experiments.

A purified 1.29 kbp fragment (Ha-MuSV_{src}) from the 5' half of the Ha-MuSV genome^{3,4}, containing the entire *src* gene, was joined to an SV40 vector such that the *src* gene was in the sense direction relative to the late transcription unit (Fig. 1). The SV40 fragment provides a functional replication origin, an intact early transcription unit, the promoter for late transcripts and the polyadenylation signal for late mRNAs, but lacks a functional late initiation triplet (AUG). This linear recombinant was transfected into secondary AGMK cells and cloned in an infectious centre assay⁸ using the SV40 early mutant tsA28 as helper virus. The linear molecules are converted into covalently closed circles by the action of cellular enzymes.

To map the template of the Ha-MuSV_{src}-specific mRNA and examine the transcript for potential splice sites, poly(A)⁺ RNA from infected cells was hybridized to ³²P-labelled DNA probes and digested with either exonuclease VII (exo VII) or S₁ nuclease. Exo VII exclusively digests single strands with free ends, whereas S₁ removes these strands as well as internal single-strand loops (due to excised RNA introns) in the DNA^{9,10}. When RNA was annealed to probe B (which represents the nucleotide sequences of the entire Ha-MuSV_{src} insert plus a short stretch of SV40 sequences at its 3' end), the major S₁-resistant band was 930 nucleotides long (Fig. 2, lanes f, g). The same heteroduplex digested with exo VII (lanes h, i) also revealed a single major band of 930 nucleotides, whereas the self-annealed DNA probe was 1,430 bp long; this strongly suggests that the Ha-MuSV_{src} sequences do not contain an intron. When probe A (which includes all Ha-MuSV_{src} sequences) was used, S₁ digestion again revealed a single major band of 930 nucleotides, indicating that no intron utilized at detectable levels is present in the region encoding the p21 protein (lanes b, c). When fragment C (which begins within the presumed coding region for p21) was used, the major S₁-resistant band is ~830 nucleotides long (lanes c', d'), suggesting that the 5' end of the

mRNA encoding p21 has been deleted from this probe and is located in the recombinant molecules within the *KpnI*-*PstI* insert ~440 nucleotides downstream (3') from the *KpnI* site.

The 5' end of the mRNA transcribed from the Ha-Mu_{src} insert was localized more precisely using a terminally labelled *HindIII* fragment which overlaps the 5' end of the gene in S₁ mapping experiments (Fig. 3). This probe should hybridize to all mRNA classes including RNA potentially initiated in SV40 sequences. Figure 3, lane *b* shows a single 160-nucleotide band, indicating that the 5' end of the Ha-Mu_{src} mRNA is located 160 nucleotides upstream from the *HindIII* site. The presence of a 5' end within the Ha-Mu_{src} insert suggests that a hitherto unknown Ha-MuSV promoter element precedes the gene encoding p21. It had been presumed that transcription of the Ha-Mu_{src} sequences proceeds exclusively from the upstream long terminal repeat (LTR)^{7,11-14}, generating the full-genome length Ha-MuSV RNA.

To demonstrate that this new promoter element is active not only in cells infected by the SVL₃-Ha-Mu_{src} recombinant, but also in cells transformed with Ha-MuSV, we analysed mRNA from Ha-MuSV-transformed C127 cells¹⁵ using the same *HindIII* probe. Figure 3, lanes *c*, *d*, shows one band which migrates at 160 nucleotides, the same position as the RNA from SVL₃-Ha-Mu_{src}-infected monkey kidney cells. This indicates that the same promoter element is also active in Ha-MuSV-transformed cells. A more prominent band of ~620 nucleotides indicates that there is a second class of RNA, the 5' end of which extends into Ha-MuSV sequences beyond those present in the probe (presumably in the LTR). As a class of RNA molecules

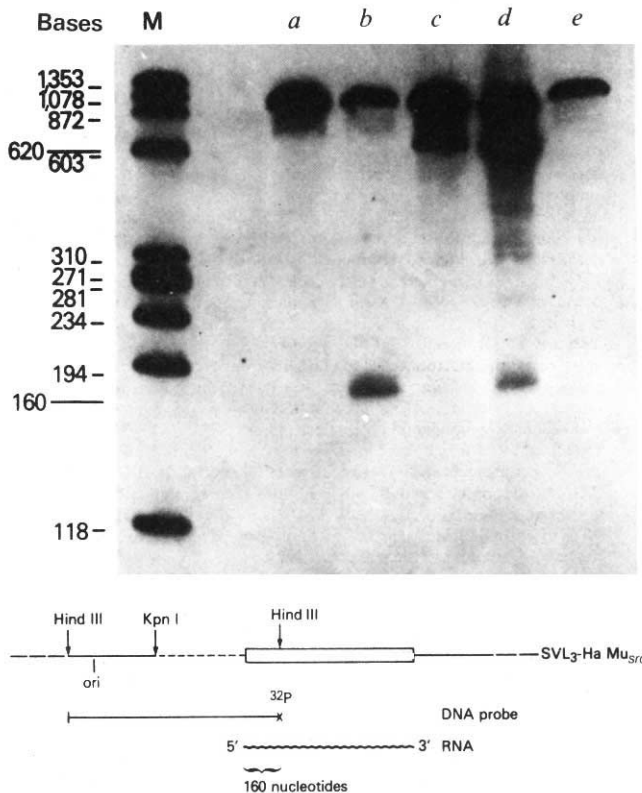


Fig. 3 Localization of the 5' end of the Ha-Mu_{src} RNA. DNA from the recombinant SVL₃-Ha-Mu_{src} was cleaved with *HindIII* and a fragment of ~920 nucleotides was 5'-end-labelled using [γ -³²P]ATP and polynucleotide kinase³¹. This probe was hybridized^{9,10} to no RNA (*a*), RNA from 2×10^6 AGMK cells infected with the recombinant SVL₃-Ha-Mu_{src} (*b*), or to RNA from C127 mouse cells transformed with Ha-MuSV, 10^7 cells (*c*) 5×10^7 cells (*d*) or RNA from 5×10^7 normal C127 mouse cells (*e*). Hybrid molecules were digested with S₁ nuclease^{9,10} and electrophoresed on a 1.4% polyacrylamide-urea gel³¹ in denaturing conditions. M, marker fragments from *HaeIII*-cleaved Φ X174 DNA.

from Ha-MuSV-transformed cells and SVL₃-Ha-Mu_{src}-infected cells exhibit the same size and 5' terminus, the 3' termini of these RNAs probably lie within Ha-MuSV sequences. We are now trying to determine the precise polyadenylation site for the SVL₃-Ha-Mu_{src} transcripts.

Viral p21 migrates in SDS-polyacrylamide gels as a doublet, the upper band of which is phosphorylated¹⁶. To establish the activity of the RNA expressed in the cells infected by the recombinant virus stock, AGMK cells were labelled with ³⁵S-methionine 72 h post-infection with SVL₃-Ha-Mu_{src}. Cell lysates were immunoprecipitated with antisera obtained from tumorous rats containing antibodies to p21. Figure 4 shows that prominent doublet bands of molecular weight (MW) 21,000 were precipitated by two different antisera (lanes 3, 4 and 5, 6), but were absent from controls precipitated from the same lysate by a normal serum (lane 2) or from a lysate of mock-infected cells precipitated with the antiserum (lane 1). The 21,000-MW doublet band resembles the doublet bands seen in a Ha-MuSV-transformed dog cell line (lane 7). Note that in addition to p21, a minor 30,000-MW band, designated p30 in Fig. 4, also seems to be specifically immunoprecipitated from the AGMK cells infected with SVL₃-Ha-Mu_{src} recombinant, but was not detected in Ha-MuSV-transformed cells (see, for example, Fig. 4 lane 7). The SVL₃-Ha-Mu_{src}-infected AGMK cells were also examined after labelling with ³²P-orthophosphate *in vivo* (Fig. 4, lanes 9, 10). A phosphorylated p21 was immunoprecipitated by the antiserum (lane 9) but not by the normal serum (lane 10). The p30 was much more heavily phosphorylated than was the p21 (based on the ³²P/³⁵S ratio). A ³²P-labelled band of MW 80,000 also reacted with the anti-p21 antiserum (Fig. 4, lane 9) but not with normal serum (Fig. 4, lane 10). This has been shown not to be SV40 T-antigen (data not presented) and its identity remains obscure.

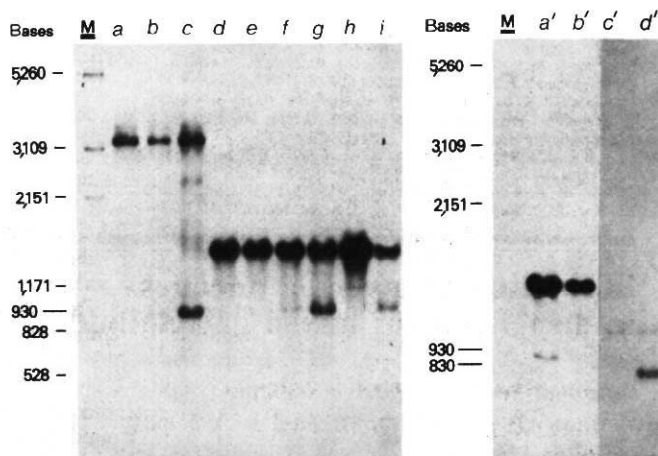
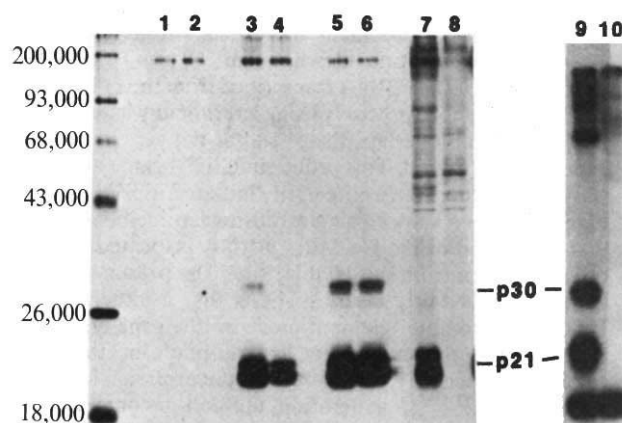


Fig. 2 Analysis of recombinant RNA molecules. Secondary AGMK cells were infected with SVL₃-Ha-Mu_{src} recombinants containing tsA28 as helper virus. Poly(A)⁺ cytoplasmic RNA was prepared 72 h after infection^{29,30}. Three different probes (A, B, C), uniformly labelled with ³²P, were prepared (see the bottom of the figure). A, the *HpaI* fragment from the recombinant molecule; B, the *KpnI*-*BclI* fragment; C, the *SmaI*-*BclI* fragment (specific activities 10^6 c.p.m. μ g⁻¹). Probe A was annealed without RNA (*a*) or with RNA equivalent to 2×10^6 cells (*b*) and 10^7 cells (*c*) followed by treatment with S₁ nuclease. Probe B was annealed without RNA (*d*), or with RNA from 10^7 cells infected with WT SV40 (*e*), from 2×10^6 cells infected with SVL₃-Ha-Mu_{src} (*f*, *h*), from 10^7 cells infected with SVL₃-Ha-Mu_{src} (*g*, *i*). The heteroduplexes were treated with S₁ nuclease (*d*-*g*) or exo VII (*h*, *i*). In the right panel, the experiment outlined in lanes *f* and *g* was repeated (tracks *b'* and *a'*, representing RNA from 2×10^6 or 10^7 cells respectively). The DNA-DNA hybrids in this experiment are more prominent. Lanes *c'*, *d'* represent the S₁ analysis of hybrids formed between probe C and 2×10^6 (*c'*) or 10^7 (*d'*) cells respectively.

Fig. 4 Immunoprecipitation of Ha-MuSV p21 from cells infected with the SV40-Ha-Mu_{src} recombinant molecules. AGMK cells in 150-cm² T flasks, 72 h after infection with SV40-Ha-Mu_{src} and tsA mutant, were labelled for 24 h with 8 ml of methionine-free Dulbecco-Vogt-modified Eagle's minimal essential medium supplemented with ³⁵S-methionine (200 µCi ml⁻¹, 1,000 Ci mmol⁻¹, Amersham) and 2% dialysed fetal calf serum. For ³²P labelling, cells were incubated in similar conditions with phosphate-free medium supplemented with 400 µCi ml⁻¹ of carrier-free ³²P-orthophosphate (Amersham). Cultures were grown at 41 °C during infection. Cell lysis and immunoprecipitation were performed by procedures previously described^{16,32} in buffer containing 1% Triton X-100, 0.5% deoxycholate, 1.0% SDS, 0.1 M NaH₂PO₄ pH 7.5, 0.1 M NaCl, 1 mM EDTA per 200 units per ml of Trasylol. 300-µl aliquots containing 5 × 10⁶ c.p.m. trichloroacetic acid-precipitable protein labelled with ³⁵S-methionine or equivalent amounts of ³²P-labelled lysates were reacted with 5 or 10 µl of either antisera containing antibodies to Ha-MuSV or control normal rat sera. The antigen-antibody complexes were precipitated by *Staphylococcus aureus* containing protein A. The precipitated proteins were analysed by discontinuous polyacrylamide gel electrophoresis in 0.1% SDS and were visualized by fluorography. Lanes 1–8 are ³⁵S-methionine-labelled extracts: lane 1, mock-infected AGMK cells with 10 µl of antiserum 1; lanes 2–6, AGMK cells infected with SV40-Ha-MuSV recombinant and precipitated with 10 µl of normal serum (lane 2), with antiserum 1 (lane 3, 10 µl; lane 4, 5 µl), or with antiserum 2 (lane 5, 10 µl; lane 6, 5 µl). Lanes 7 and 8, Ha-MuSV-transformed MKCK dog cell extracts were treated similarly with 10 µl of antiserum 1 (lane 7) or 10 µl of normal serum (lane 8). The fluorographic film exposure in lanes 7 and 8 exceeded that in lanes 1–6, and therefore the intensity of p21 bands does not reflect the relative amounts of the p21 protein in these cells. Lanes 9 and 10 represent ³²P-labelled AGMK cells infected with the SV40-Ha-Mu_{src} recombinant reacted with 10 µl of antiserum 1 (lane 9) or 10 µl of normal serum (lane 10).



A major class of RNA containing discrete 5' termini mapping within the Ha-MuSV portion of the recombinant molecules suggests that a promoter for the Ha-Mu_{src} RNA is located upstream from its transcriptional initiation site. This transcript may be independent of the distant LTR but depend on the presence of certain SV40 activating sequences in the SV40 recombinant molecules^{17,18}. An unrecognized promoter element (such as a Goldberg-Hogness box) might directly precede the sequences of the gene encoding p21. Preliminary sequence analysis (R. Dhar, personal communication) has revealed evidence for such a sequence preceding the newly described Ha-MuSV 5' ends by the expected distance. Recent studies have shown that the presence of the LTR greatly increases the efficiency of cell transformation^{7,12,14}. If the *src* gene contains its own promoter sequence, one might speculate that the LTR could enhance transformation by increasing the frequency or specificity of integration, and/or by increasing the amount of p21-specific mRNA through positioning 5' RNA ends directly before the p21-encoding gene.

The results of the S₁ and exo VII nuclease analyses suggest that the mature Ha-Mu_{src} RNA molecules are unspliced. Although certain mRNAs seem to require splicing for their stability^{19–21}, other genes such as histones, interferon and some viral genes (refs 22–26 and C. Weisman and A. Ullrich) seem to give rise to colinear unspliced transcripts. In relation to this concept, certain viable late SV40 deletion mutants seem to generate variable but significant quantities of unspliced late 19S mRNA²⁶, and deletion of the single intron from the rat preproinsulin 1 gene in SV40-rat preproinsulin recombinants does not abolish the production of stable rat insulin transcripts²⁷. Thus, there seem to be at least two basic mechanisms for mRNA biogenesis. Further studies with the SVL₃-Ha-Mu_{src} recombinant molecules may help to clarify the complex relationship between splicing and mRNA stability.

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Formation of inverted micelles in dispersions of mixed galactolipids

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Several recent studies^{1–5} have reported particles in freeze-fracture replicas of mixed phospholipid dispersions. The precise molecular arrangement of the particles is, however, still unknown^{6,7}. We have previously shown that similar particles occur in dispersions of mixed *sn*-3- galactosyldiacylglycerols⁸. We demonstrate here that the particles seen in galactolipid mixtures correspond to inverted lipid micelles sandwiched within a membrane bilayer.

Lipid mixtures favouring the formation of particles usually contain one lipid, that when dispersed alone in water forms a lamellar phase, together with another lipid that forms an hexagonal phase. We used mono- and digalactosyldiacylglycerols mainly because X-ray diffraction studies show that the former forms hexagonal II-type structures in water, whereas the latter forms lamellar structures^{9,10}, but also because they account for ~75% of the total membrane lipids of the chloroplast¹¹.

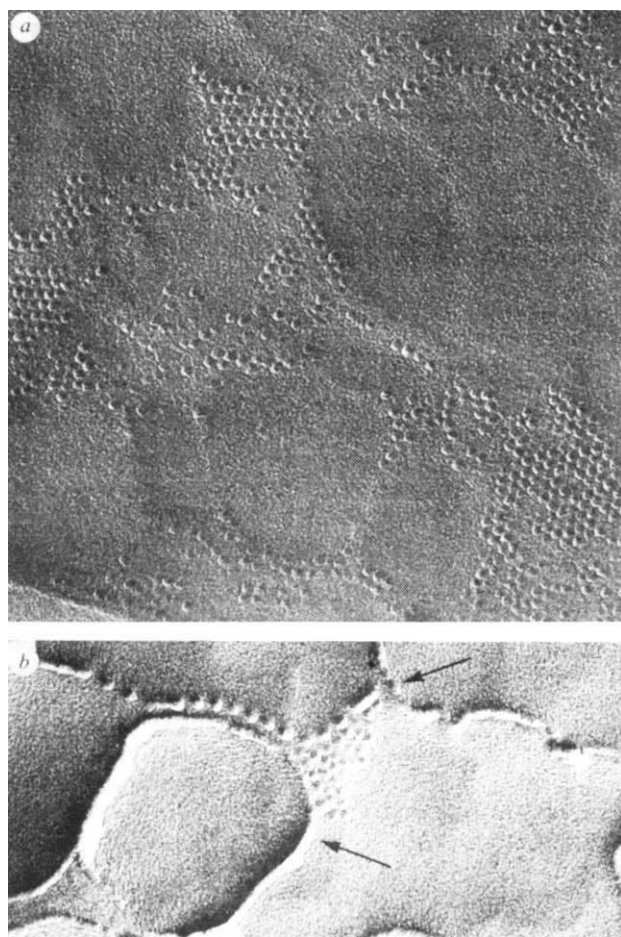


Fig. 1 Electronmicrographs ($\times 125,000$) of freeze-fracture replicas prepared from sonicated dispersions of mono- and digalactosyldiacylglycerols mixed in a molar ratio of 2:1. *a*, Area containing large numbers of freeze-fracture particles organized into arrays. *b*, Example of one of the tubular structures (arrowed) often seen in these preparations that seems to have broken down to form a string of particles. The lipids were purified from bean leaves¹⁰, mixed in solvent and dried under vacuum. The dry lipid mixture was then dispersed in oxygen-free distilled water by sonication. Samples were quenched from 20°C in a slurry of nitrogen and fractured at -115°C in a Polaron freeze-fracture device.

Typical freeze-fracture replicas obtained from sonicated dispersions of 2:1 mixtures of mono- and digalactosyldiacylglycerols (the ratio found in chloroplast membranes) are shown in Fig. 1. The lipids tend to form aggregates of liposomes, the membranes of which often contain large numbers of particles (10–11 nm diameter) organized into planar arrays (Fig. 1*a*). Suspension in salt solutions (100 mM univalent or 10 mM divalent cation) reduces the particle diameter to 8–9 nm. The arrays are usually hexagonal close packed, but square or oblique packing is also observed. Tube-like structures, often associated with strings of particles, are also common (Fig. 1*b*).

We find, in agreement with Miyazaki *et al.*¹², that ordered structures are also found in negatively stained preparations of mixed galactolipids. A typical electronmicrograph is shown in Fig. 2. The main feature (area A) is highly ordered, with a main periodicity of ~ 10 nm. The build-up of stain and the moiré effects observed in some areas suggest that the structure is several layers thick. Area B shows a region where this structure appears to break down, yielding a set of hexagonally packed tubular structures with a diameter very similar to the main repeat distance of the central array. Area C seems to correspond to a more conventional liposomal-type structure. The repeat distance of 7.0–7.5 nm is, however, much greater than the

normal thickness of a lipid bilayer and each repeating unit seems to have a partially stained central region.

Figure 3*a* shows an optical diffraction pattern, taken from area A in Fig. 2, indexing on a near-rectangular lattice with spacings along the two axes of ~ 7.1 and 10.4 nm. Close examination of the original object (Fig. 3*b*) indicates that it arises from two superimposed near-rectangular arrays of stain deposits displaced with respect to each other by half the main inter-particle repeat distance. We interpret these arrays as arising from a cross-sectional view of stacked sheets of lipid bilayers, each containing a close-packed array of inverted micelles (see Fig. 4). One array, probably the more heavily stained, is thought to correspond to end-on views of continuous aqueous channels between the stacked bilayers, and the other to end-on views of inverted micelles packed in rows within the bilayers. The possibility that the inverted micelles also fuse to form tubes cannot be excluded. On this basis, the repeat distance within the plane of bilayers (7.1 nm) corresponds to the thickness of two lipid monolayers (~ 4.0 nm) plus the diameter of the aqueous compartment of an inverted micelle (~ 3.5 nm), and the repeat distance perpendicular to the bilayers (10.4 nm) to the thickness of two bilayers plus the diameter of an aqueous compartment.

The diameter (~ 10.5 nm) of the tubular structures seen in area B and the width (~ 7.5 nm) of the structures seen in area C are consistent with the idea that they correspond to cross-sectional views of tubular liposomes and less ordered stacks of inverted micelle-containing lipid bilayers of the type seen in area A, as shown in Fig. 4. Lucy and Glauret¹³ have reported the occurrence of similar tubular structures in dispersions of lecithin and cholesterol which were thought to consist of micelles. The

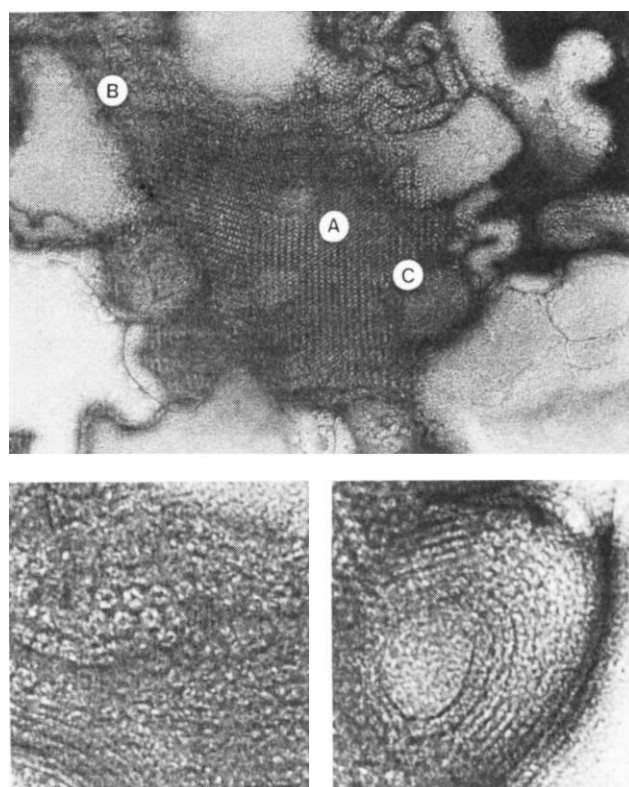


Fig. 2 An electronmicrograph ($\times 87,500$) of a negatively stained preparation of a similar dispersion to that used in the freeze-fracture studies. Samples were stained with uranyl acetate (1%) on an electron microscope grid. Samples subsequently washed with distilled water showed no staining, which indicates that no positive staining occurred. Area A consists of a large, highly ordered structure. Areas B and C ($\times 275,000$), shown at the bottom of the figure, correspond to an end-on view of tubular liposomes (left) and a cross-sectional view of a liposomal-type structure (right) respectively.

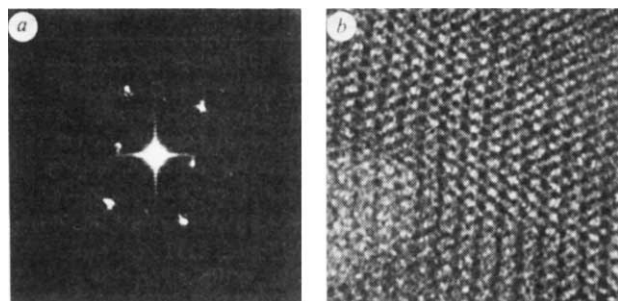


Fig. 3 *a*, Optical diffraction pattern of area A of the electron-micrograph shown in Fig. 2, recorded using the optical diffractometer described elsewhere¹⁶. The pattern shows a near-rectangular symmetry with repeat distances of 7.1 and 10.4 nm in the two principal orthogonal planes. *b*, Enlarged ($\times 275,000$) view of the area from which the diffraction pattern was taken.

fact that monogalactosyldiacylglycerol, the main component of our dispersions, tends to take up an hexagonal-II structure suggests that such an arrangement is extremely unlikely in this case.

The very highly ordered appearance of the lipids in the negatively stained samples raises the possibility that the stain itself might be affecting the organization of the lipids but freeze-fracture replicas of stained and unstained samples showed no appreciable differences. We found, however, that addition of ethylene glycol greatly increased the order seen in such replicas (see Fig. 5). Area A shows a region where the lipid is organized into a quasi-crystalline structure; area B shows a liposome-type structure in which similar (~ 14 nm diameter) particles appear to be embedded in the membrane. The increased particle size presumably reflects differences in the balance between the hydrophobic and electrostatic interactions of the lipids. The presence of similar particles in what seems to be quasi-crystalline aggregates of inverted micelles and in distinct lamellar phases strongly supports the idea that the particles seen in freeze-fracture replicas of untreated samples and in the negatively stained preparations are indeed inverted micelles.

The role of ethylene glycol seems to be to induce the formation of new particles rather than to improve the preservation of preformed particles. Cullis *et al.*¹⁴ have proposed that inverted micelles may play an important part in such cellular functions as

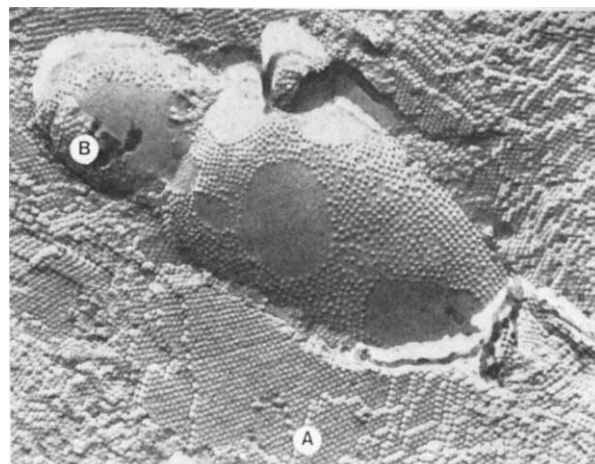


Fig. 5 Electronmicrograph ($\times 60,000$) of a freeze-fracture replica obtained from a 2:1 molar ratio mixture of mono- and digalactosyldiacylglycerols suspended in a 58% solution of ethylene glycol. Sample preparation was as described in Fig. 1 legend. Area A shows a region where the lipids seem to be organized into a quasi-crystalline structure made up of inverted micelles. Area B shows a region where similar particles are embedded in the membrane of a liposomal structure.

membrane fusion, exo- and endocytosis, and the transbilayer movement of lipids. Our observation that ethylene glycol, which promotes fusion both of liposomes and cell membranes¹⁵, leads to a substantial increase in the number of freeze-fracture particles in dispersions of mixed galactolipids is consistent with this view.

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Corrigendum

In the article 'Gravitational focusing and the association of distant quasars with foreground galaxies' by C. R. Canizares, *Nature* **291**, 620–624, there was a numerical error in the analysis of the Seldner and Peebles data, which caused a significant underestimate of the number of 'true' quasar-galaxy associations needed to explain their results. A corrected analysis gives $\sim 100 \pm 40$ true associations and an estimated mean enhancement factor for quasar surface density $\bar{\zeta}_{sp} \sim 0.9 \pm 0.4$ (with no allowance made for the inevitable systematic errors). This enhancement factor is several times larger than the estimate of $\bar{\zeta} \sim 0.14$ for gravitational focusing by galaxy halo stars using nominal parameters. However, $\bar{\zeta}$ values ≥ 0.5 are within the plausible range of parameters for massive halos, so although the correspondence between the Seldner and Peebles data and the prediction of the gravitational focusing calculation is weakened, the basic thesis of the paper remains unchanged. Further, more refined tests are required. Also, the integrand in the expression for N_e should be $w_{gg}(\delta) d\Omega(\delta)$ (this is not the source of the numerical error). The author thanks Rachel Webster for bringing to his attention the possibility of an error.

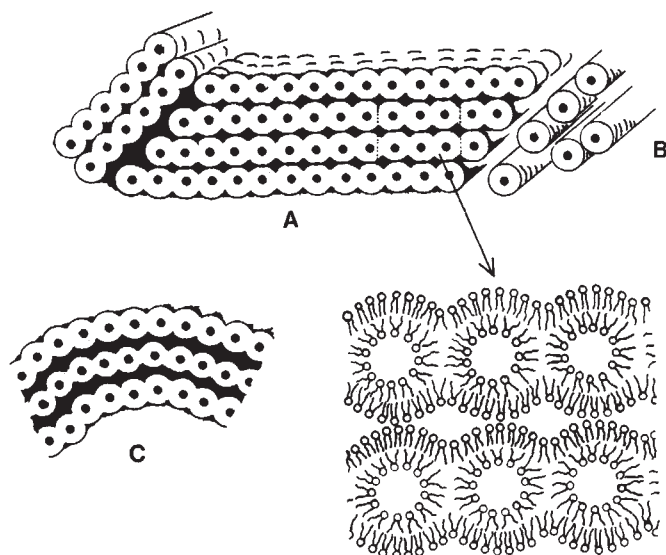


Fig. 4 The proposed organization of lipids giving rise to the structures seen in areas A, B and C of the electronmicrograph shown in Fig. 2. An expanded view of the molecular arrangement of the inverted micelle-lipid bilayer sandwich, which is thought to serve as the basis of these structures, is also shown. (See text for details.)

MATTERS ARISING

Did Taupo's eruption enhance European sunsets?

A NEW precise date of 186 AD has been postulated¹ for a series of large rhyolitic plinian and pyroclastic flow eruptions at Taupo, New Zealand. Evidence came from Chinese and Roman records of a brilliant red Sun and Moon and of clouds dimming the Sun. Although the Taupo eruption was one of the largest within the period AD 100–200, there is evidence that this eruption did not disperse enough fine tephra into the atmosphere, let alone into the Northern Hemisphere, to produce the phenomena described.

Evidence suggesting that the Taupo eruption and the recorded Sun phenomena are unrelated is as follows: (1) The Chinese excerpt¹ describes the Sun and Moon themselves as being "red as blood" when they rose and set and do not describe glow phenomena or "sunset colours". Direct red colouration of the Sun followed the eruptions of Krakatau 1883 and Bali 1963 and was generally restricted to near the volcanic source and to areas at about the same latitude². Red colouration of the Sun is due to very small particles ($<0.5 \mu\text{m}$) or smoke haze scattering short-wavelength light. Thus the Chinese record is more consistent with atmospheric dust or smoke haze in the lower troposphere than higher-altitude volcanic dust. Similarly, one Roman account of a widespread red colour is also more consistent with atmospheric dust or smoke.

(2) Although the Taupo eruptions were large, they were not as exceptional as implied by Walker³. I have recalculated the volume of the "Taupo pumice fall deposit" to be 14 km^3 , in contrast to the 24 km^3 calculated by Walker³, who claimed that ~70% of the volume (16 km^3) was sub-2 mm particles that had all fallen beyond land: this is excessive, as it is based on an incorrect assumption of crystal liberation and fine ash generation in the plinian eruption column³. Furthermore, if 16 km^3 of fine ash had been generated, then a more extensive distribution of this tephra should have been found in offshore cores^{4–6}. I deduce a more realistic figure of 20% of fine ash. (3) The height reached by the eruption column is controlled by convective strength and heat output, not total eruption volume⁷. The total column density can never be greater than surrounding atmosphere density, as with zero shear strength the column would then collapse. Thus 16 km^3 of fine ash could not rise beyond 50–55 km (ref. 7) and no large quantities could reach the mesosphere. At these heights, particles would fall to lower

elevations within a few weeks², before the widespread dispersal claimed by Wilson *et al.*¹ could occur. Thus Lamb's deduction² of material reaching the Northern Hemisphere only if erupted north of 20°S latitude is considered valid. For comparison, an eruption on Bali (8.5°S) in March, 1963 produced an observed column up to 50 km high which had fallen to 31–35 km by November 1963, when 'sunset phenomena' were observed from Australia to England². Particles with diameters of $\sim 1 \mu\text{m}$ were being concentrated in the equatorial region at 20–22 km up to April 1964⁸. Thus the Taupo material would also be expected to fall rapidly from $\sim 55 \text{ km}$ height to the lower stratosphere and, being erupted from 39°S , would spread over the Southern Hemisphere. As large quantities of fine ash could not have reached the Northern Hemisphere, the chances of this material causing red Sun phenomena witnessed by Europeans are slight.

(4) Confirmation of the Taupo date could be sought in polar ice cores¹. Acidity profiles in the Greenland ice sheet⁹ are incomplete for between AD 0 and 500. In the Greenland ice cores, an earlier and larger eruption from Taupo (Waimihia Tephra, $1640 \pm 130 \text{ BC}$)^{3,10} has not been noted, so the Taupo event may not be represented either. Consequently, migration of volcanic acids and hence micro-particles into the Northern Hemisphere following the Taupo eruption is doubtful. (5) Finally, and most importantly, Wilson *et al.* quoted a date for only one of the Roman accounts (AD 186) and a range of AD 168–189 for the Chinese record. They did not attempt to substantiate that the three accounts were describing the same event. Thus the ancient writers were probably describing different events, possibly the results of dust-storms or fires.

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WILSON *ET AL.* REPLY—We answer Froggatt's five objections to our interpretation¹ as follows:

(1) We accept that the red Sun, Moon and sky colouration would have been caused by the finest particles (mostly $\leq 0.5 \mu\text{m}$)² and that particles from sources other than Taupo could have been responsible. We believe that a close temporal correlation exists between optical effects recorded by Chinese and Roman historians, and a volcanic eruption which seems to be one of the outstanding explosive eruptions of the past 2 millennia. The optical phenomena are consistent with the arrival in the Northern Hemisphere of significant quantities of the finest particles from the Taupo eruption.

(2) We stand by our figure for the volume of the Taupo ultraplinian pumice-fall deposit, and cannot comment on Froggatt's estimate as we do not know its basis. However, the absolute volume of ash released to the atmosphere is much less important in determining the dispersal than the height to which the material is injected. Regarding the apparent absence of significant amounts of Taupo eruptive material in offshore sediment cores, two of the three studies quoted were in areas well north³ and well south⁴ of the seaward extension of the mapped dispersal axis. The third offshore study⁵ was centred $\geq 1,600 \text{ km}$ downwind from source where the expected thickness of Taupo eruptive material would be $<1 \text{ mm}$; because of bioturbation, such an ash layer is unlikely to survive as a discrete bed^{6,7}.

(3) We have never claimed that the eruption column height was controlled by the eruption volume, or that 16 km^3 of fine ash rose higher than 50–55 km. However, two methods indicate that the height of the ultraplinian eruption column was 50 km or more (ref. 8 and L. Wilson, personal communication). Material of a grain size appropriate to cause the atmospheric effects recorded in the Northern Hemisphere, if injected to 55 km height, might be expected to take up to several years to fall even to 30 km (ref. 9) and would not fall significantly "within a few weeks". The 1963 eruption of Mt Agung was a much smaller and less violent even than the Taupo eruption; its maximum eruption column height was only $\sim 13 \text{ km}$ and the first 3 months' activity produced only 0.28 km^3 of material¹⁰.

(4) We agree that the bulk of the ash resulting from the Taupo ultraplinian phase, as well as from earlier and later explosive phases of the same eruption, would probably have been confined to the southern hemisphere. This, however, does not preclude the dispersal of significant amounts of the finest particles to the Northern Hemisphere. Regarding the

1. Wilson, C. J. N., Ambraseys, N. N., Bradley, J. & Walker, G. P. L. *Nature* **288**, 252–253 (1980).
2. Lamb, H. H. *Phil. Trans. R. Soc. A* **226**, 425–533 (1970).
3. Walker, G. P. L. *J. volcan. geotherm. Res.* **8**, 69–94 (1980).
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9. Hammer, C. U., Clausen, H. B. & Dansgaard, W. *Nature* **288**, 230–235 (1980).
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apparent absence of an acidity anomaly for the Waimihia eruption (dated at ~3,800 yr BP (ref. 11)), we note that the Greenland ice-core study¹², from the period 3,050–4,650 yr BP, considered only acidity levels greater than 2.6 $\mu\text{equiv H}^+$ per kg of ice. As the far larger Northern Hemisphere eruptions of Santorini and Mt Mazama only produced acidity signals of 5.3 and 6.0 $\mu\text{equiv H}^+$ per kg of ice, respectively, then the absence of a marked peak attributable to the Waimihia eruption is not significant. The dispersal of its deposits, moreover, implies that the Waimihia eruption was appreciably less powerful than the Taupo eruption.

(5) Froggatt summarily dismisses the historical evidence and concludes that the observed phenomena were possibly the results of fires or dust storms. However, internal evidence suggests that none of these alternatives seems likely, although the probability of one of the early writers describing the effects of a supernova¹³ cannot be ruled out.

In conclusion, none of Froggatt's objections invalidate the grounds on which we proposed a correlation between the Taupo eruption and the optical effects observed in ~AD 186 in both China and Rome.

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Does mate choice occur in *Drosophila melanogaster*?

PARTRIDGE¹ recently reported the results of a series of experiments purporting to demonstrate that "...one component of offspring fitness can be increased by mate choice in *Drosophila melanogaster*". Her experiments involved the mating of females to single males and to a choice of males. The data show a significant difference in the competitive ability of offspring from choice mating when compared with those from no-choice matings ($P < 0.02$).

Her paper, entitled "Mate choice increases a component of offspring fitness in fruit flies", implies a conclusive result but she admits that the results can be interpreted in two ways. First, individual females "...could then mate with a fly that was successful in some sort of competition with members of its own sex". We believe that Partridge must recognize that the occurrence of male-male competition could mask any possible choice by females. Her second alternative is that "flies may be able to detect heritable fitness in members of the other sex". The choosing by females of 'fit' males is commonly referred to as mate choice or female choice^{2,3}.

The problem lies in Partridge's central question: "Does mate choice increase a component of fitness (competitive ability of larvae) in *Drosophila melanogaster*?" This question is, by definition, composed of two parts: (1) does mate choice occur? and (2) how does this affect offspring fitness? Part (1) must be demonstrated before any conclusions can be drawn relating offspring fitness to mate choice.

Partridge has demonstrated that either adult males that are more successful in male-male competition have larval offspring which are better competitors (heritability of a fitness component), or that mate choice by females of maximally fit males occurs. As her experiments were not designed in such a way as to distinguish between the two alternatives of male-male competition and mate choice, we believe that she has not justified her claim that mate choice is occurring in *D. melanogaster*.

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PARTRIDGE REPLIES—The central criticism by Kingett *et al.* is that they say I stated¹ that female choice of males as mates must be responsible for my results. In reply, I quote the last paragraph of my paper: "The result could be produced by various genetic mechanisms. First, fitness may indeed be heritable, possibly as a consequence of mutational load. If this were the case, the results could be produced in two ways. (1) Fitter flies may be better at detecting or obtaining access to mates. Members of the other sex could then mate with a fly that was successful in some sort of competition with members of its own sex. (2) Flies may be able to detect heritable fitness in members of the other sex. Fitter flies could then be actively chosen as mates. Both possibilities would suggest that all flies should prefer the same sorts of mates. Alternatively, flies with high levels of heterozygosity may have high fitness. In this case, flies might produce fitter offspring by mating with individuals genetically unlike themselves, so that their offspring will have higher levels of heterozygosity. This last possibility would imply that genetically dissimilar flies would show different patterns of mate choice".

Possibility (1) encompasses the alternative suggestion of Kingett *et al.* that inter-male competition could be responsible for my results. In their final paragraph, Kingett *et al.* state that my results must be produced by heritable fitness variation in males. This is not necessarily true, as my final suggestion in the above quotation makes clear.

I used the term 'mate choice' in the title and introduction of my paper to avoid the cumbersome alternative 'The opportunity to mate non-randomly increases a component of offspring fitness in fruit flies' which is what my results demonstrate.

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1. Wilson, C. J. N., Ambraseys, N. N., Bradley, J. & Walker, G. P. L. *Nature* **288**, 252–253 (1980).
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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

BOOK REVIEWS

Science in farming: fifty years of the ARC

J.C. Bowman

FIFTY years in being is good reason for any organization to have a celebration. For the UK Agricultural Research Council it is also cause for well-deserved congratulations, especially from the farming industry worldwide and from scientists in other organizations. As part of the ARC's activities to mark its Golden Jubilee it has published *Agricultural Research 1931-1981*, and for this too it deserves congratulation.

There are two main themes to the book. The first five chapters, written by the immediate past Secretary to the ARC, Sir William Henderson, deal with the foundation of agricultural research since 1700, with the establishment of the ARC in 1931 and with its development since then. The important determinants of the success of an organization — the terms of reference, the people, the structure and the relationship with other organizations — clearly emerge from Sir William's account.

The ARC did not have an easy birth nor has its life always been smooth; for instance, the relationship of the ARC with the Agricultural Departments appears to have been uneasy. In 1931 the ARC was charged only with providing criticism and advice on agricultural research. In 1935 the Council, in spite of opposition from the Agricultural Departments, gained the right to carry on agricultural research itself and from then has expanded its research capability both in its own institutes and in university-based units and departments. The money for the ARC programme at that time was provided from the Vote for Scientific Investigation. In 1971 came the Rothschild proposals and the implementation in the following year of the customer-contractor principle between government departments and research councils. Money was transferred from the Science Vote to the Agricultural Departments for them to commission research appropriate to farming's need from the ARC and other bodies.

Now, about 60 per cent of the money spent by the ARC on agricultural research is provided by the Agricultural Departments. The ARC seems to have had a hard task in establishing the need and advantage of having an agricultural research programme independent of the control of the Agricultural Departments and in close touch with the farming industry. Throughout the 50 years the Agricultural Departments seem to have wished to take over the ARC and to act as

Agricultural Research 1931-1981: A History of the Agricultural Research Council and a Review of Developments in Agricultural Science During the Last Fifty Years. Edited by G.W. Cooke. Pp.367. ISBN 0-7084-0180-5. (Agricultural Research Council, 160 Great Portland Street, London W1: 1981.) £23.

spokesmen for the farming industry. Sir William says it is too early to judge the effect of the Rothschild proposals but concludes that

The research policy for agriculture must be to enlarge scientific knowledge so as to provide the options for meeting the changing social, economic and political situations as they arise in the future. The achievement of this objective demands the fostering of good science within centres of excellence.

Many will agree with these views and will go on to ask whether the substantial role of the Agricultural Departments in determining the level of expenditure and the programme of research is appropriate. Sir William's history of the ARC provides a sound basis from which to consider such questions.

The second main theme in the book, complementary to the history of the Council, is a review of the research carried out and the contribution it has already made to changes in farming throughout the world. This part is written by 20 contributors, including Dr R. Riley the present Secretary to the Council and many of the

ARC Institute Directors. The style is surprisingly consistent and the accounts interesting — a credit to the editor Dr G. W. Cooke. Not only do they highlight the way scientific investigation, initially with little apparent application, has subsequently changed the practice and the economics of farming but they also emphasize the international nature of science. The emergence of major farming innovations from the careful collation of small areas of knowledge, produced by research workers in several parts of the world, is a repeated story in crop breeding, animal nutrition, agricultural engineering and other fields recounted in this book.

The appeal of this book will be to readers of several kinds. To those interested in the development of public scientific organizations and the interplay with other public institutions of learning and administration this is a useful case study. (Although it is a pity, for historians, that Sir William Henderson did not list the main sources of his information.) To those interested in the ARC's scientific contribution to farming it is an excellent record of achievement with plenty of scope for economists to engage in post-mortem cost-benefit analyses. To those interested in a story of how scientists work and make progress possible, it is an inspiration. □

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Revisionist zeal for Restoration science

J.Z. Fullmer

Science and Society in Restoration England. By Michael Hunter. Pp.233. ISBN hbk 0-521-22866-2; ISBN pbk 0-521-29685-4. (Cambridge University Press: 1981.) Hbk £18.50, \$37.50; pbk £5.95, \$12.95.

HISTORIOGRAPHIC study reveals that for no other period in English history has scholarly debate been as vigorous as it has been for Restoration England. The period was crowded with incident, chronicled by participants and observers equipped with lively pens and livelier minds. Social, economic, political, theological, scientific stresses, beliefs and events divide historians' attention, just as they divided

Restoration society. Michael Hunter's book reflects that turbulence and diversity of the Restoration scientific community and surveys the ensuing historical discussions. In one sense, each of his seven chapters (Restoration Science: its Character and Origins; The Significance of the Royal Society; The Scientific Community; Utility and its Problems; Politics and Reform; Science, Learning and the Universities; Atheism and Orthodoxy) focuses in turn on an aspect of the historians' controversies. Hunter's announced aim was

to use a close reading of manuscript and printed sources to show how Restoration science related

to contemporary society in terms of support and apathy, facilities and impediments, motivation and reservations [p.6].

He achieved his goal, not by study of the internal development of the separate sciences, but by emphasizing the social integration of the scientific elements of the Restoration. Of course, and quite properly, it is the newly founded Royal Society which dominates his work, just as the early Fellows appear to have dominated the Restoration scientific community.

The book concludes with an appendix, where Hunter shows that the famous frontispiece for Thomas Sprat's *History of the Royal Society* was actually prepared for another, and related, work by John Beale. Unfortunately for future readers, the hardback version of Hunter's work places the reproduction on the dust jacket. Since dust jackets rarely survive on books in a library, the impact of Hunter's appendix will be lessened unless subsequent readers take the trouble to seek out Sprat's work.

Hunter's "Bibliographical Essay" will guide the reader to it. This essay is distinguished by its clear topical organization, mirroring that of the chapters, and by its extensive coverage of the literature. Any scholar drawn to Restoration history or Restoration science will find the essay an exceedingly useful guide. Readers of Hunter's text will do well to consult it before they begin the text, for only here is there a clue to the idiosyncratic numbering system used within the footnotes. Even with the help of both the essay headnote, and of the index which follows it, readers will not easily break into Hunter's code.

While this final section contains statement enough to permit a reader to decide whether or not to pursue a particular reference, Hunter's text usually provides detailed criticisms of individual works. Often he announces the central problem his chapter will treat, and then proceeds to review what others have said about it. In the course of this sort of discussion Hunter offers the evidence for accepting or rejecting earlier interpretations, but it should be pointed out that his emphasis is clearly on rejection, or, at best, conditional acceptance of previous works. Thus, in the chapter Politics and Reform, Hunter's announced aim is to discover if "the new science in seventeenth-century England [can] be associated with any particular religious or political ideology" (p.113). The "Merton thesis", for example, he finds wanting because Merton's definition of "Puritan" is so vague that Merton's conclusions become indisputable. Invoking Latitudinarianism as the binding force for the Restoration scientific community is likewise dismissed. Hunter argues that it is neither Puritanism nor Latitudinarianism which characterizes Restoration scientists, but the homogeneity of Puritan political and religious beliefs. He thinks that the difficulty of detecting affinities between

religious outlooks and "scientific" outlooks is paralleled in the search for affinities between "scientific" outlook and political outlook. Political affinities he finds best analysed by examining not the "actual [political] practices of scientists", but their

ambitions, their hope that the principles and methods of the new philosophy, if applied to the economy and society, would bring about improvements of wide importance [p.117].

Hunter discovers clues to these aspirations in some of the millenarianist dreams, in schemes for technological improvements, in projects for a "new", "universal" language based on rational principles, in reforms of basic units of measurement and in educational reforms. But especially important, Hunter thinks, is Sir William Petty's introduction of "political arithmetic". He concludes, finally, that Restoration science indeed had a political flavour, which permeated all scientific endeavours.

Hunter's critical and corrective zeal moves him in every paragraph. So exuberantly does he argue that often his sentences get out of hand. Vague pronoun references, and occasional wordings which make no sense, mar his splendid and spirited performance. (The first sentence on p.62 is an obvious example, but see also that on p.82 describing the work of Towneley's circle. The sentences on p.109 typify his personal pronoun usage: in describing the work of the two Casaubons he leaves the reader confused about who wrote what.) Such deficient editorial control is regrettable, and readers should be warned they will often find the text unnecessarily hard to read. Yet, despite the difficulties, Hunter's work is a good if controversial introduction to Restoration science for graduate students in the history of science and in the history of the Restoration. □

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A new bible for carotenoid biochemists

Brian H. Davies

The Biochemistry of the Carotenoids, 2nd Edn. Vol.1, *Plants*. By T.W. Goodwin. Pp. 377. ISBN 0-412-21690-6. (Chapman & Hall: 1980.) £27.50.

THIS is the first single-author book devoted exclusively to the biochemistry of the carotenoid pigments to have appeared in some 30 years, that is since T.W. Goodwin's *The Comparative Biochemistry of the Carotenoids* was published by Chapman & Hall in 1952. The present work, in spite of a different title, is described as the second edition of that earlier book and is but the first volume of a pair, being subtitled *Plants*; the second will deal with animal carotenoids. That the title has changed is appropriate to a new and different approach, for the book is not merely an updated version of the original. Nor could it be, for the intervening three decades have seen too many changes for that.

The development of collections of pure cultures of microorganisms and the successes of geneticists in producing mutants of plants of all kinds have both contributed to the wealth of living material now available for study by the carotenoid biochemist. The same period has also witnessed developments in chromatography (TLC and HPLC) as a means of purifying individual carotenoids from complex biological extracts. Many carotenoid identifications of the 1950s were based on empirical spectroscopic and chromatographic comparisons with known pigments which, as recently as 15–20 years earlier, had confessed their structures to the classical degradative approaches of Karrer and his pupils. But now, in the 1980s, the final

elucidation of the structures (and stereochemistry) of much smaller samples of new (and old) carotenoids is best left to those experienced in specialized techniques of physico-organic analysis (MS, NMR, CD and so on) which are, themselves, developments of the past three decades.

A consequence of all these advances is that while Goodwin originally gave a fully comprehensive account of some 90 then-recognized carotenoids, he has now been faced with the task of describing the structures, distribution, function and biosynthesis of over 500 such pigments. It is little wonder that his title and approach have had to change (he admits to having become more selective, though I am aware of nothing of any importance that he has omitted), or that the book now has to appear as two separate volumes.

The importance of using the newer physico-organic analytical methods is emphasized in Chapter 1 ("Nature and Properties"). Their potential for providing unambiguous structures is succinctly explained in a way easily comprehensible even to those with little formal chemical training. His more detailed treatment is reserved for those techniques (absorption spectroscopy, chromatography, chemical tests) which are more readily available in biological laboratories and which yield crucial (but not always unambiguous) information on carotenoid structure. Tentative structures thus obtained, really have to be confirmed by physico-organic methods available in specialized laboratories.

Those who may mourn the loss of the word "comparative" from the title need

not distress themselves, for the approach of the new book is still essentially one of comparison. After two excellently-written general chapters on biosynthesis and on function, the author moves on to consider, separately, all aspects of the carotenoids of the photosynthetic tissues of seed-bearing plants, and of those of their reproductive tissues and roots. Then he deals, again in separate chapters, with the carotenoids formed by mosses, liverworts and spore-bearing vascular plants, by algae, by fungi, by the non-photosynthetic and by the photosynthetic bacteria. For each group of organisms, a consideration of distribution is followed by a description of those features of carotenoid function and biosynthesis which are peculiar to that group, thus supplementing the earlier description of common features. The control of carotenogenesis by genetic, environmental and chemical means is discussed whenever appropriate.

Many carotenoid biochemists (and others) have tried to use correlations of (a) carotenoid structure and distribution with (b) plant classification as a retrospective comment on how plant evolution may have taken place. Goodwin's final chapter, "Biogeochemistry", describes the few results but considerable potential of carotenoid analysis as a means of demonstrating how plant evolution actually occurred.

This really is an excellent book; it is not only scholarly and written with great clarity, but also well planned. The basic structures of some 194 carotenoids are included, among other line illustrations, in addition to a number of apposite electron micrographs. But perhaps one of the most important features of the book is the number and quality of the comparative tables — 86 of them. Of these, the most impressive covers seven pages and shows the distribution of 67 fruit carotenoids throughout the 160 higher plant species examined to date. Fully referenced as it is, it could stand on its own as a review article. Also packed into the book's 377 pages are a general index and a particularly useful species index.

Although several excellent books on carotenoids and other plant pigments have appeared over the past decade, Goodwin's new volume is clearly set to be the most authoritative, comprehensive and comprehensible work available on their biochemistry. There are few really readable reference works on any discipline, so those of us with an interest in carotenoids are particularly fortunate. I am sure this book will stimulate even more interest in carotenoid biochemistry, for there is much in it to appeal to the intellect and imagination of undergraduates, research workers and practitioners in all areas of biological science. □

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How do electrons get from here to there?

Thomas J. Meyer

Electron Transfer Reactions. By R.D. Cannon. Pp.351. ISBN 0-408-10646-8. (Butterworth: 1981.) £32, \$84.95.

COMPARED to most chemical reactions, electron transfer is a simple process. An electron hops from one chemical site to another, in many cases without significant change in structure. Even so, electron transfer is at the heart of many critical issues in chemistry, physics and biology, and its study is currently enjoying a remarkable vitality. As evidence note the book *Tunnelling in Biological Systems* edited by B. Chance *et al.* (Academic Press, 1979) which provides an account of a meeting on the same theme held four years ago in Philadelphia.

The origins of this new interest in electron transfer are twofold. The first is the existence of a useful interplay between theory and experiment which is leading to a "solution" of sorts — a solution in the sense that the microscopic factors controlling rates of electron transfer reactions can be understood by chemists, biochemists and physicists based on well-established ideas in chemistry and physics. The second is the spill-over effect that these advances will and are having on related problems in chemistry, physics, biology and photochemistry.

Having said all of this, it must be added that the subject can be a source of extreme frustration. For a chemist, much of the theory — which has its origins in work on defects in solids, radiationless processes or nonequilibrium thermodynamics — is barely comprehensible. Also, there are far too many experimental results and yet too few critical experiments, the result being that attempts to generalize often lead to confusion.

Into this setting comes *Electron Transfer Reactions* by R.D. Cannon. The first thing to be said about the book is that it is a real work of scholarship. A diverse and voluminous literature is brought together in a single volume. The second is that it is a book by a chemist written for other chemists. There are introductory chapters on electron transfer in the gas phase and in the solid state, but the emphasis is on electron transfer in solution, mostly in aqueous solution and predominantly involving transition metal ions.

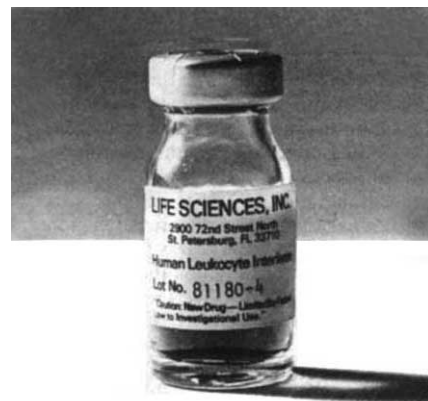
Cannon's synthesis of the available experimental data is a creative act, and much thought has been given to both their organization and interpretation. The result is generally good — for example the introduction of specimen calculations, the systematization of such diverse topics as

free energy profiles, multiple electron steps and successor complexes — but occasionally bad — the use of symbols instead of real examples to illustrate reaction types is sometimes hard to follow, and the development of the theoretical background is somewhat inconsistent and confusing. The book includes chapters on reaction pathways and mechanisms, theory, electron transfer through ligand bridges and optical electron transfer, in addition to the chapters on electron transfer in the gas phase and the solid state.

One limitation is that no attempt is made to develop the emerging generalities alluded to above. For example, there is little mention of either photochemistry or of electron transfer in biological systems. It is also true that the author is occasionally guilty of focusing on the bizarre or the unusual at the expense of the more general. However, I find that Cannon's book serves admirably in its role as a sophisticated guide to electron transfer in solution. It is a valuable and badly needed addition to the literature, since its predecessor, *Mechanisms of Electron Transfer* by Reynolds and Lumry (The Ronald Press, 1966), is now well out of date. □

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Richard Mabey's *The Common Ground* (for review see *Nature* 286, 87) has recently appeared in paperback. The book is published by Arrow and costs £1.95.

CORRESPONDENCE

Continued from page 422

smallest in Scotland. Founded by Professor Ronnie Bell FRS, in 1967, it survived the loss of Professor Willie Parker, to continue as a well-known, well-equipped, highly cost effective unit in one of Britain's modern universities.

Faced with a considerable reduction in resources, we have compared our performance across a broad spectrum of activities with other chemistry departments in Scotland.

- (1) We have the smallest number of academic staff.
- (2) We have produced, over the last eight years, the largest number of publications per member of staff.
- (3) We are ranked second in terms of the value of SRC grants currently in operation per member of staff. At the last round of SRC grant considerations, all five of the department's applications were funded by the Chemistry Committee.
- (4) We have a student/staff ratio of 8.6/1 which is the average for Scotland.
- (5) Our entrance standards are about average for Scottish universities, and chemistry applications have increased by 37 per cent in 1981 over 1980 (which in turn were higher than previously).

We, therefore, find ourselves questioning the actions of the University Grants Committee which appear to be inconsistent with criteria they themselves have outlined, and which, if put into effect at Stirling, will put our future at risk.

R.M. CLAY, J.M.G. COWIE, B.G. COX,
R.W. HAY, H. MASKILL, P. MURRAY-RUST,
A.E.A. PORTER, F.G. RIDDELL,
J.S. ROBERTS, W.V. STEELE, I.C. WALKER
Chemistry Department,
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Academics' year

SIR — G. W. Brindley's suggestion (*Nature* 27 August, p.791) of a 9-month academic year contract as the solution to the financial crisis in UK universities is ludicrous. In the United States, in actual practice, academics are paid as much for a nominal 9-month academic year as for a 12-month year, the only difference being that they are now free to pay themselves an additional salary from their research grant.

The 9-month academic year is thus nothing more than a convenient fiction that enables principal investigators to divert research funds into their own pockets, effectively diminishing total support for research, while enhancing their own standard of living.

ROBERT J. YAES

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Effluent safety limits

SIR — Day and Cross¹ have recently reported very interesting data on the *in situ* production of ²⁴¹Am from its parent ²⁴¹Pu in the Irish Sea which receives radioactive effluents from the Windscale nuclear fuel reprocessing facility. When authorizing the quantities of radioactivity which may be discharged by Windscale, the United Kingdom regulating agencies [Department of the Environment, Ministry of Agriculture, Fisheries and Food (DOE/MAFF)] have published only limited

information^{2,3} on the environmental ingrowth of the radiotoxic alpha-emitter ²⁴¹Am from the unrestricted discharges of ²⁴¹Pu. The publication of much more comprehensive data and calculations by Day and Cross show that the annual *in situ* formation of ²⁴¹Am has been 480 per cent, 240 per cent and 265 per cent of those quantities of ²⁴¹Am discharged directly from Windscale in 1977, 1978 and 1979 respectively. Their calculations show further that if the recent rates of ²⁴¹Pu discharge are maintained, the *in situ* ²⁴¹Am production will continue to rise from ~600 Ci per year at present to achieve a steady state situation of ~1,300 Ci per year ingrowth after the turn of the century.

Although the DOE/MAFF-authorized maximum direct discharge limit of alpha activity to the Irish Sea is 6,000 Ci per year (by the Windscale pipeline) it is not clear from what has been published whether the DOE/MAFF limit includes the fact that there is an increasing ingrowth of ²⁴¹Am which under steady state conditions will generate a further ~1,300 Ci of alpha activity per year. In other words, does the radiological assessment lead to the definition of the safe total annual input as being 6,000 Ci or 7,300 Ci alpha activity? If the total acceptable annual input is thought to be 6,000 Ci, then the pipeline alpha discharge limit should be continuously revised downward to 4,700 Ci per year if similar ²⁴¹Pu discharges are to be maintained.

The authorized annual alpha discharge limit set for the Windscale pipeline was raised to 6,000 Ci in 1970. Since then some significant data have been reported on the behaviour of plutonium and americium isotopes in this coastal environment. (1) These substances are now known to be accumulated very effectively in local sediments around Windscale^{2,4}, and their dispersion and dilution by seawater are much less than was previously expected. (2) It has been shown that the transport of these radionuclides within these sediments is slow^{5,6}. (3) The ingrowth of ²⁴¹Am in the sediments is an increasingly substantial proportion of the alpha activity¹.

The result of these effects is that the Windscale alpha activity is not widely diluted but is concentrated in a small portion of the environment. Day and Cross's data and these other observations should thus be taken into account in the regulation of the discharges of alpha activity and ²⁴¹Pu from Windscale in the future. It is interesting and probably optimistic to note that British Nuclear Fuels Limited reduced the unrestricted discharge of ²⁴¹Pu from Windscale by 49 per cent in 1980⁷.

S.R. ASTON

Department of Environmental Sciences,
University of Lancaster, UK

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4. Livingston, H.D. & Bowen, V.T. *Nature* **269**, 586 (1981).
5. Aston, S.R. & Stanners, D.A. *Nature* **289**, 581 (1981).
6. Aston, S.R. & Stanners, D.A. *Envir. Pollut. Ser. B* (in the press).
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Notes from a Recorder

SIR — All seventeen Recorders must have been flattered by the comment (*Nature* 3 September, p.1.) that they provide "virtually the only intellectual continuity" in the British Association for the Advancement of Science. Indeed, it makes a welcome change from being mistaken for either a member of the judiciary or a musical instrument.

However, some of the problems you outline which face the British Association are being tackled.

To even the most casual observer, the bimodality of the age structure of BA must be apparent. Young people — British Association Young Scientists (BAYS) — attend and so too do scientists of more mature years. But scientists of middle years go to specialist conferences (if they can obtain funding at all). Although this latter group may be persuaded to break into family holidays to give a non-expenses paid paper, they are often unable to stay the whole week. And the meeting is the poorer. Closer links with relevant professional and learned societies and planning of joint lecture sessions might bring back these scientists to the BA. They may, of course, not wish to come. It is often more comfortable to be surrounded by a coterie using familiar, insulating jargon.

If this approach is only partially successful, the BA may yet attract this middle age range group by another means. Provision of nursery school facilities and programmes for the whole family on lines well established at many university summer schools should prove more attractive to scientists with young families. A start on this scheme will be made by the Programme Planning Committee for implementation at Liverpool in 1982; by 1983 at Sussex a whole range of activities for family groups should be available.

All the topics listed in the *Nature* leading article as worthy of BA consideration have been aired in the last four years, one, genetic engineering, in my own Section D (zoology) last year at Salford. I would suggest also that the BA is the right forum for popularizing science. It is certainly not easy, especially with the advent of superb scientific documentaries on television; both natural history museums and the BA have learnt this to their cost.

However, to see the light of recognition dawning on the faces of young audiences when excellent speakers are putting across difficult concepts leads one to suppose that the BA can, and does, popularize rather well. Excellent proof of this was available at the Section D session at York this year on predator/prey relationships.

Perhaps where we do need to adjust our sights is in reviving the feeling of excitement generated by the announcement of discoveries and breakthroughs at the annual meeting. These are notoriously difficult to produce to order, but not impossible to stage-manage provided one chooses an innovative and productive section president.

TONY FINCHAM

(Chairman of
Recorders Committee)

British Museum (Natural History),
London SW7, UK

BOOKS RECEIVED

Mathematics

- ASIMOW, L. and ELLIS, A. J. *Convexity Theory and its Applications in Functional Analysis*. London Mathematical Society, Monographs 16. Pp.266. ISBN 0-12-065340-0. (Academic: 1980.) £23.20, \$56.
- DE MOTTONI, P. and SALVADORI, L. (eds). *Nonlinear Differential Equations. Invariance, Stability and Bifurcation*. Proceedings of a Symposium held in Villa Madruzzo, Trento, Italy, May 1980. Pp.357. ISBN 0-12-508780-2. (Academic: 1981.) \$22.
- HAIGHT, F. A. *Applied Probability. Mathematical Concepts and Methods in Science and Engineering*, Vol. 23. Pp.290. ISBN 0-306-40699-3. (Plenum: 1981.) \$35.
- LEITMANN, G. *The Calculus of Variations and Optimal Control: An Introduction. Mathematical Concepts and Methods in Science and Engineering*, 24. Pp.311. ISBN 0-306-40707-8. (Plenum: 1981.) \$35.
- ZIEGLER, Z. (ed.). *Approximation Theory and Applications*. Proceedings of a Workshop on Approximation Theory and Applications Technion, Haifa, Israel, May-June 1980. Pp.358. ISBN 0-12-780650-4. (Academic: 1981.) \$26.

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- BEN-SHAUL, A., HAAS, Y., KOMPA, K. L. and LEVINE, R. D. *Lasers and Chemical Change*. Pp.497. ISBN 3-54010379-1. (Springer-Verlag: 1981.) DM 85, \$40.50.
- BERNASCONI, J. and SCHNEIDER, T. (eds). *Physics in One Dimension*. Proceedings of an International Conference, Fribourg, Switzerland, August 1980. Pp.368. ISBN 3-540-10586-7. (Springer-Verlag: 1981.) DM 65, \$34.20.
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- FITTING, D. W. and ADLER, L. *Ultrasonic Spectral Analysis for Nondestructive Evaluation*. Pp.354. ISBN 0-306-40484-2. (Plenum: 1981.) \$45.
- HARMUTH, H. F. *Nonsinusoidal Waves for Radar and Radio Communication*. Pp.396. ISBN 0-12-014575. (Academic: 1981.) \$45.
- HILL, J. W. *Introductory Physics*. Pp.116. Flexi ISBN 0-333-24442-7. (Macmillan Education, Basingstoke: 1980.) £1.75.
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- KAHNG, D. (ed.). *Silicon Integrated Circuits. Part A, Applied Solid State Science*. Pp.416. ISBN 0-12-002954-5. (Academic: 1981.) \$66.50.
- MARTON, L. and MARTON, C. (eds). *Advances in Electronics and Electron Physics*, Vol. 55. Pp.403. ISBN 0-12-041655-X. (Academic: 1981.) \$62.
- NARASIMHAMURTY, T. S. *Photoelastic and Electro-Optic Properties of Crystals*. Pp.514. ISBN 0-306-31101-1. (Plenum: 1981.) \$37.50.
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- CLARKE, M. J. *et al.* (eds). *Metal Complexes. Structure and Bonding*, Vol.44. Pp.202. ISBN 3-540-10494-1. (Springer-Verlag: 1981.) DM 96, \$50.40.
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- MOORE, T. C. *Research Experiences in Plant Physiology: A Laboratory Manual*. 2nd Edn. Pp.348. Flexi ISBN 3-540-90606-1. (Springer-Verlag: 1981.) Np.
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Applied Biological Sciences

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- KNAPP, W. (ed.). *Leukemia Markers. Proceedings of the Conference held in Vienna, February 1981*. Pp.600. ISBN 0-12-416750-0. (Academic: 1981.) £20, \$48.

General

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